Table I. Santa Rosa Plum Components

1. Acetaldehyde	19. 1-Pentanol	37. 1-Octanol
2. Methanol	20. Ethyl butyrate	38. 1-Heptyl acetate
3. Ethanol	21. 1-Butyl acetate	39. Linalool
4. Ethyl ether (solvent)	22. 2-Methyl-2-pentenal	40. Benzyl alcohol
5. 1-Propanol	23. 3-Methyl-1-butyl acetate	41. Benzyl acetate
6. Ethyl acetate	24. 2-Methyl-1-butyl acetate	42. 2-Phenylethanol
7. Chloroform	25. trans-3-Hexen-1-ol	43. Ethyl octanoate
8. 2-Methyl-3-buten-2-ol	26. cis-3-Hexen-1-ol	44. α-Terpineol
9. 2-Methyl-1-propanol	27. 1-Hexanol	45. Ethyl phenylacetate
10. 1-Butanol	28. trans-2-Hexen-1-ol	46. γ-Octalactone
11. 1-Penten-3-ol	29. 1-Pentyl acetate	47. 1-Methylnaphthalene
12. 1-Propyl acetate	30. Benzaldehyde	48. γ-Nonalactone
13. 1,1-Diethoxyethane	31. 1-Heptanol	49. Ethyl decanoate
14. 3-Hydroxy-2-butanone	32. 1-Butyl butyrate	50. Biphenyl
15. 3-Methyl-1-butanol	33. Ethyl hexanoate	51. Ethyl anisate
16. 2-Methyl-1-butanol	34. cis-3-Hexenyl acetate	52. γ-Decalactone
17. 2-Methyl-1-propyl acetate	35. 1-Hexyl acetate	53. Butylated hydroxytoluene
18. Diethyl carbonate	36. γ-Hexalactone	

tivity of each data source to small isomeric differences decreases in general, and the possibility of error increases. In all cases listed in Table I, the mass spectral and relative retention time data are fully consistent with the assignments made.

Most of the compounds found are fairly common fruit volatiles, with the possible exception of ethyl anisate. Acetate esters predominate, but appreciable quantities of the higher γ-lactones appear as well. Chloroform is used in our laboratory for syringe cleaning, so it is likely an artifact. Similarly, butylated hydroxytoluene is a preservative in the ether solvent used, so it is not a plum constituent.

Some effort was made to correlate the presence of certain components with the fruit's characteristic aroma. The gc column effluent was sniffed (thermistor detector), impressions of several judges were noted, and a number of test mixtures were subsequently prepared, using the gc chromatogram as a guide to the relative concentrations of the components. However, because of the seasonal nature of plums, fresh fruit material was not available for more formal and systematic evaluation of the components' aroma contributions.

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Autoxidation of Some Constituents of Hops. I. The Monoterpene Hydrocarbon, Myrcene

Robert H. Dieckmann and S. Rao Palamand*

Controlled degradation of myrcene, the major monoterpene hydrocarbon of hop oil, is discussed with special emphasis on the role of myrcene as a precursor for the formation of flavor compounds occurring in naturally aging hop oil. Products are isolated and identified by means of gas chromatographic, mass spectral, and infrared analyses. Related possible degradation pathways are suggested for a number of these second- and third-order terpenoid constituents, and spectral data for several terpene alcohols, oxides, ketones, and polymerization products are reported.

The study of the essential oil of hops (0.5–1.0% w/w) has been of importance to the brewing industry for many years, as hops constitute one of the major raw materials involved in the brewing cycle, contributing to both the odor and flavor of the finished beer. Only in the last 15 years, however, has gas chromatography, and later, mass spectrometry, given the analyst a tool which is capable of separating and analyzing the complex mixture of over 200 compounds. Results have been published by, among others, Buttery et al. (1964), Jahnsen (1963), and Roberts

(1962). Of this mixture the main components of the fresh oil were found to be terpene hydrocarbons, specifically the monoterpene myrcene, and the sesquiterpenes β-caryophyllene and humulene (Buttery and Ling, 1967; Jahnsen, 1963).

Upon storage, however, DeMets and Verzele (1968) and Kowaka and Hashimoto (1967) reported that the oxygenated fraction of the oil shows a rapid build-up, with a concomitant loss of myrcene.

The purpose of this study, therefore, is to determine what changes are produced by short storage at elevated temperatures in a number of these hop oil components. Our experimental findings are presented for the first of these compounds, the monoterpene, myrcene.

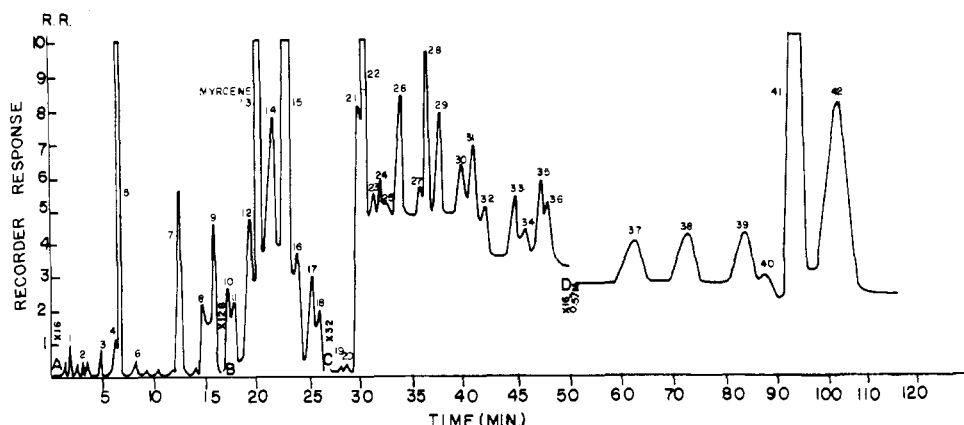Technical Center, Anheuser-Busch, Inc., St. Louis, Missouri 63118.

Figure 1. Gas chromatogram of myrcene reaction mixture autoxidized at 65° for 48 hr.
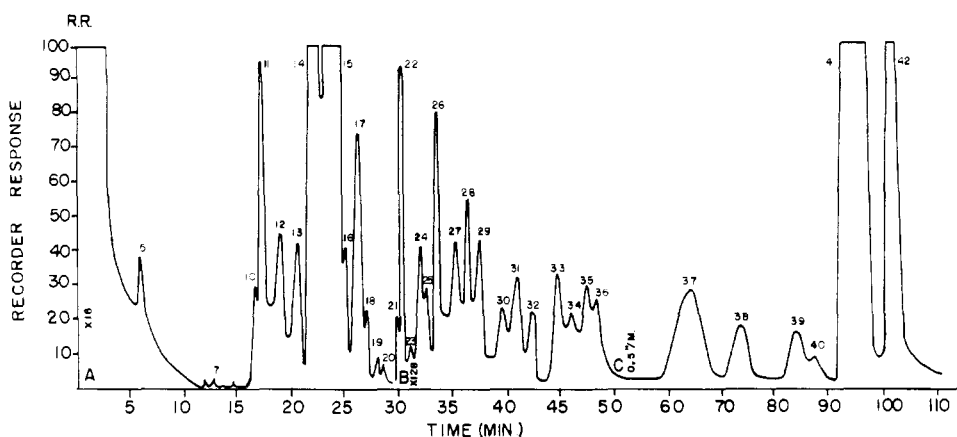

Figure 2. Gas chromatogram of myrcene reaction mixture autoxidized at 100° for 48 hr.

EXPERIMENTAL SECTION

Myrcene Purification. Technical grade myrcene (K&K) was purified by silica gel chromatography according to the method of Jahnsen (1963), followed by gas chromatography of the hexane eluent residue using a 12 ft × 0.5 in. stainless steel prep column packed with 20% Carbowax 20M on 60–80 mesh Chromosorb P. Purity of the collected myrcene was verified on the experimental analytical column.

Autoxidation of Myrcene. (1) Purified myrcene was subjected to a 48-hr degradation at 65° by vigorous magnetic stirring in the presence of air. Aliquots were removed at 8-hr intervals for analysis by gas chromatography. (2) In order to study the effect of temperature upon the autoxidation process, the thermal conditions of the reaction were raised to 100°, and a cold water condenser was attached to prevent serious loss of product through evaporation. Aliquots were again withdrawn at 8-hr intervals for 48 hr and analyzed by gas chromatography.

Gas Chromatography. All autoxidized samples were analyzed on an F&M 810 dual column instrument equipped with a flame ionization detector. A 4 mm × 20 ft column coated with 20% Carbowax 20M on 60–80 mesh Chromosorb P was employed for experimental analysis and connected to the detector via a 10:1 effluent splitter. The oven temperature was programmed linearly from 60 to 195° at 4°/min, and held at the upper limit for 65 min. The carrier flow was measured at the detector at 40 cm³/min. Individual peaks were collected as they eluted in thin-wall capillaries immersed in liquid nitrogen, and subsequently rinsed from the tube with diethyl ether. In
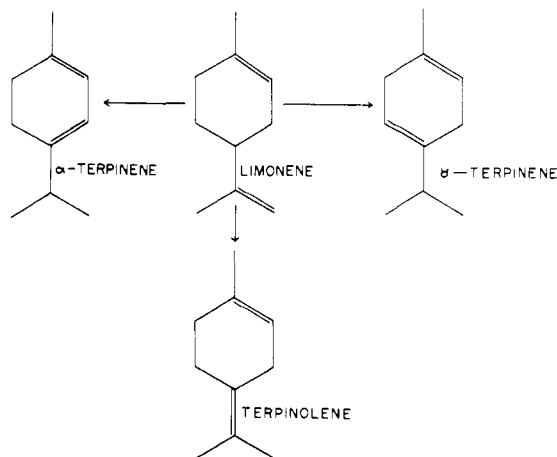

Figure 3. Suggested pathway for identified monocyclic reaction products of myrcene autoxidation.

this way, multiple collections facilitated data assimilation on many minor components.

Identification of Products of Autoxidation. The identification of component peaks was accomplished by the use of gc retention data on two dissimilar columns and infrared and mass spectrometry. All gc identifications include the spiking of the sample with authentic compounds to check the homogeneity of the peak in question.

Infrared spectra were run on a Perkin-Elmer 457 instrument using KBr disks and a beam condenser. All mass

Table I. Identification of the Products of Autoxidation of Myrcene

Peak no.	Identification	Method	Ir (wave number)	Mass spectra (m/e)
1	Diethyl ether	Gc		
2	Isoprene	Gc		
3	Isobutyraldehyde	Gc		
4	Propanal	Gc, ms		M$^+$ 58
5	Acetone	Gc, ms		M$^+$ 58, 43 (100)
6	Butanal	Gc		
7	2-Methyl-3-buten-2-ol	Gc		
8	Tricyclene	Gc, ms		M$^+$ 136, 93 (100), 121 (24), 79 (24), 41 (24), 92 (22), 91 (22), 77 (22), 136 (16), 39 (16), 94 (13)
9	α-Pinene	Gc, ms, ir	3036, 890; 790; 1385, 1375, 1368	M$^+$ 136, 93 (100), 92 (31), 91 (30), 77 (30), 79 (26), 41 (26), 39 (21), 121 (17), 43 (17), 105 (15)
10	Camphene	Gc, ms, ir	3070, 1660	M$^+$ 136, 93 (100), 121 (90), 79 (75), 67 (73), 41 (88), 55 (53), 39 (50), 77 (50), 107 (40), 91 (40)
11	8(9)-p-Menthene	Ms		M$^+$ 138, 69 (100), 41 (83), 70 (20), 39 (18), 53 (13), 27 (12), 82 (11), 67 (11), 95 (8), 55 (8)
12	β-Pinene	Gc, ms, ir	3080, 1640, 878; 1388, 1370	M$^+$ 136, 93 (100), 41 (69), 69 (52), 79 (37), 77 (35), 39 (32), 91 (28), 27 (24), 121 (17)
13	Myrcene	Gc, ms, ir		
14	α-Terpinene	Gc, ms, ir	1668, 835	M$^+$ 136, 93 (100), 91 (42), 79 (37), 77 (35), 136 (25), 121 (22), 43 (22), 119 (18)
15	Limonene	Gc, ms, ir	3080, 1640, 890; 1380	M$^+$ 136, 68 (100), 67 (52), 93 (48), 41 (34), 39 (34), 53 (32), 79 (32), 27 (25), 77 (18), 94 (18)
16	γ-Terpinene	Gc, ms, ir	1668, 840 (w)	M$^+$ 136, 93 (100), 91 (53), 121 (33), 77 (31), 136 (29), 41 (29), 92 (21), 43 (20), 79 (20)
17	p-Cymene	Gc, ms, ir	1510, 1382, 1365, 810	M$^+$ 134, 119 (100), 91 (32), 41 (30), 134 (27), 79 (21), 39 (20), 93 (20), 77 (19)
18	Terpinolene	Gc, ms, ir	1375, 1360	M$^+$ 136, 93 (100), 91 (67), 79 (61), 77 (60), 41 (55), 39 (45), 43 (42), 121 (41), 53 (39)
19	2-Methyl-2-hepten-6-one	Gc		
20	Alloocimene	Gc		
21	trans-α-Pinene oxide	Gc, ms		M$^+$ 152, 119 (100), 91 (68), 134 (59), 120 (43), 117 (39), 41 (38)
22	cis-α-Pinene oxide	Gc, ms		M$^+$ 152, 119 (100), 91 (71), 134 (63), 120 (50), 117 (39), 41 (31)
23	Thujone	Gc		
24	Linalool oxide	Gc, ms		M$^+$ 154, 59 (100), 43 (72), 55 (60), 68 (58), 41 (49), 94 (38), 93 (38), 111 (29), 67 (27)
25	Linalool oxide	Gc, ms		M$^-$ 156, 59 (100), 43 (68), 55 (58), 68 (52), 41 (43), 94 (39), 93 (35), 111 (35), 67 (31)
26	Linalool	Gc, ms		M$^+$ 154, 41 (100), 71 (95), 43 (81), 93 (68), 69 (64), 55 (63), 27 (60), 39 (51), 53 (48), 67 (42)
27	Camphor	Gc, ms		M$^+$ 152, 95 (100), 41 (91), 81 (83), 69 (61)
28	α-Terpineol	Gc, ms		M$^+$ 154, 59 (100), 93 (86), 43 (81), 121 (77), 81 (50), 41 (48), 136 (45), 45 (41), 79 (39)

Table I (*Continued*)

Peak no.	Identification	Method	Ir (wave number)	Mass spectra (m/e)
29	Unidentified			M^+ 152, 41 (100), 69 (52), 39 (20), 27 (20), 55 (17), 29 (15), 43 (15), 67 (15), 79 (11), 53 (10)
30	Unidentified			M^+ 152, 41 (100), 91 (75), 55 (73), 92 (58), 43 (58), 79 (53), 39 (50), 119 (48), 83 (43)
31	Citral	Gc, ms		M^+ 152, 69 (100), 41 (85), 39 (36), 84 (33), 94 (30), 109 (27), 67 (23), 95 (19), 79 (17)
32	Myrtenal	Ms		M^- 150, 79 (100), 41 (96), 107 (64), 39 (50), 91 (50), 77 (48), 43 (44), 69 (39), 67 (37), 55 (33)
33	Carvone	Gc, ms, ir	3080, 1635, 890; 1670; 1380	M^- 150, 82 (100), 41 (55), 54 (49), 39 (45), 93 (40), 108 (31), 27 (30), 53 (28)
34	Nerol	Gc, ms		M^+ 154, 41 (100), 69 (83), 71 (81), 70 (35), 93 (31), 39 (25), 55 (21), 43 (19), 53 (19)
35	Unidentified			M^+ 152, 41 (100), 107 (79), 91 (58), 55 (55), 84 (53), 93 (52), 39 (51), 43 (50), 79 (45)
36	Geraniol	Gc, ms		M^+ 154, 41 (100), 69 (100), 68 (31), 39 (29), 53 (22), 27 (20), 55 (17), 93 (15), 81 (15)
37	Unidentified			M^- 152, 68 (100), 67 (82), 79 (81), 41 (73), 29 (73), 93 (59), 91 (55), 55 (55), 39 (45), 53 (36)
38	Unidentified			M^+ 272, 69 (100), 41 (77), 91 (29), 93 (29), 67 (27), 55 (23), 79 (23), 81 (21), 105 (21), 119 (21)
39	Unidentified			M^+ 272, 69 (100), 41 (77), 93 (41), 55 (33), 91 (22), 43 (21), 67 (21), 79 (19), 105 (15), 119 (14)
40	Unidentified			M^+ 232, 41 (100), 69 (88), 93 (65), 91 (38), 55 (31), 67 (31), 79 (31) .
41	*m*-Camphorene	Gc, ms		M^+ 272, 69 (100), 41 (90), 91 (28), 93 (21), 119 (18), 79 (18), 67 (18), 105 (16), 109 (15)
42	*p*-Camphorene	Gc, ms		M^- 272, 69 (100), 41 (75), 93 (57), 67 (29), 91 (27), 55 (25), 109 (22), 79 (21)

spectra were recorded on a Varian Matt 111 Gnome gc-mass spectrometer at 80 eV. A 12 ft × 2 mm 8% FFAP column was employed for the rechromatography of the collected samples, ensuring purity of the mass-scanned peak, and elimination of the water problem associated with liquid nitrogen collections.

Most compounds were identified by direct comparison of their infrared and/or mass spectra with those obtained from authentic standards. For those standards which were unavailable, published literature data were substituted.

α- and γ-terpinene were synthesized by the silica gel isomerization of terpinolene at 150° according to the method of Hunter and Brogden (1963). *m*- and *p*-camphorene were synthesized by the Diels–Alder reaction of myrcene under a slow stream of nitrogen at 200°. Purification of the reaction products was accomplished by gas chromatography and analysis by mass spectrometry under the conditions already given.

RESULTS AND DISCUSSION

The chromatograms of the autoxidized myrcene samples under both experimental procedures are reproduced in Figures 1 and 2. Reaction product identifications are listed in Table I along with the method of identification and pertinent spectral data. While some peaks are as yet unidentified, the experimental data obtained seem to be sufficient to enable a delineation of the four separate reaction mechanisms as illustrated in Figures 3–7 and discussed below.

Cyclization. The appearance of limonene, α-terpinene, γ-terpinene, and terpinolene (Figure 3) in significant yield seems to prove the ability of myrcene to cyclize to the *p*-menthadiene skeletal structure. As limonene is the main reaction component, it seems likely that the appearance of the terpinenes and terpinolene is due to secondary rearrangements of the exocyclic double bond. These findings are in agreement with previous work done on the rel-
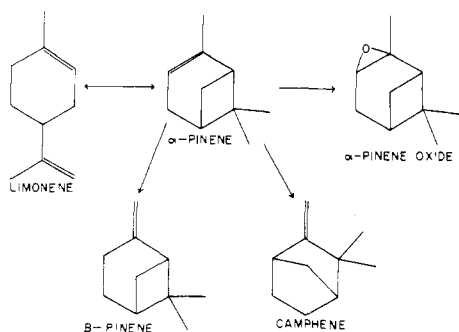
Figure 4. Suggested pathway for identified bicyclic reaction products of myrcene autoxidation.
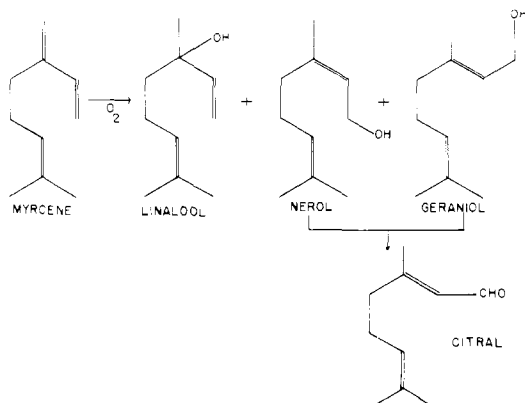

Figure 5. Air oxidation products of myrcene.

Figure 6. Air oxidation products of limonene.

Figure 7. Suggested pathway for identified disproportionation products of limonene.

Figure 8. Rate of polymerization of myrcene at different temperatures.

ative reactivity of p-menthadienes by Arbuzov and Isaev (1953), Hunter and Brogden (1963), and Watnabe (1962).

The appearances of large amounts of camphene and β-pinene likewise support the theory of a secondary cyclization involving both the endocyclic and exocyclic double bonds of limonene. The appearance of only small amounts of α-pinene in the reaction mixture was puzzling, in that α-pinene would have to be the intermediary between limonene and the other bicyclic products. However, the identification of α-pinene oxide, coupled with the known relative isomerization rates of α-pinene and limonene (Watnabe, 1962), lead to the proposed reaction scheme shown in Figure 4. α-Pinene is formed from limonene, but, being more reactive than its precursor, is either rearranged to β-pinene or camphene, epoxidized to α-pinene oxide, or reverted to the precursor, limonene.

Oxidation. Autoxidation of monoterpenes has long been considered to proceed through the reaction intermediates of peroxides and epoxides, which are subsequently opened to form the alcohol, and further oxidized to the corresponding ketone. Oxidation in the reaction mixture is proven by the presence of α-pinene oxide, linalool oxide, and trace amounts of an unidentified dioxide of M+ 168 (not shown in Table I due to insufficiency of accumulated data). However, except for α-pinene oxide, the intermediates were too unstable under existing experimental conditions and too low in concentration to allow a thorough analysis. The secondary products, on the other hand, were much more stable, and both the alcohols and ketones resulting from oxide openings were detected in the sample. Linalool, citral, geraniol, and nerol are products of air oxidation on myrcene directly (Figure 5), while α-terpineol and carvone result from the oxidation of the primary product, limonene (Figure 6). One is tempted to consider the dioxide of limonene to be the unidentified component of M+ 168, but insufficient data have been accumulated to identify it as such. Likewise, carveol must be the other product of epoxide opening, which is then further oxidized
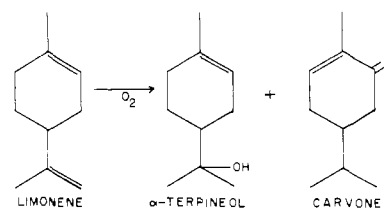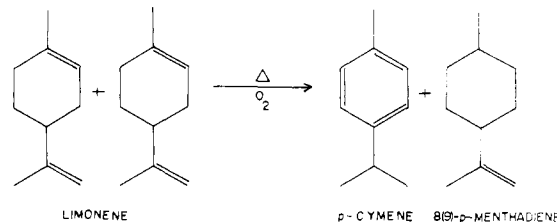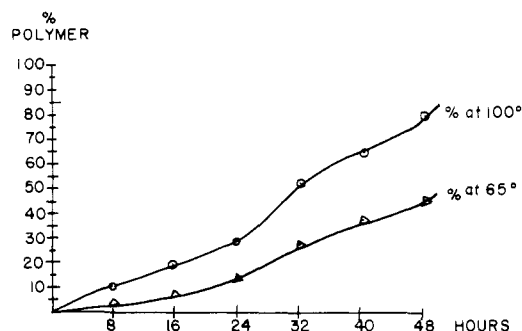
to carvone. However, carvone and carveol are poorly separated by the gc conditions employed and although a small amount of another monoterpene alcohol (M+ 152) was present in addition to nerol in the collected carvone, the data were insufficient to positively establish its identity as carveol. Other as yet unidentified oxidation products are still under investigation, and gc retention data suggest that some may be products of α-pinene oxide ring openings.

Disproportionation. The appearance of large quantities of p-cymene in the reaction mixture was at first thought to be a product of the secondary oxidation of the p-menthadiene primary products. However, the isolation of a peak corresponding to 8(9)-p-menthene leads to the conclusion that limonene is undergoing the disproportionation reaction seen in Figure 7, yielding p-cymene and the menthene as primary products. The participation of terpinolene and α-terpinene in the reaction is ruled out due to the absence of both 3-p-menthene and 1-p-menthene in the reaction mixture as described by Eschinazi and Pines (1956).

Polymerization. The late end of the gas chromatogram is characterized by several unresolved peaks which are mixtures of at least three components. Rechromatography of the collected peaks at 175° and analysis by mass spectrometry disclosed that the main components of peaks 41 and 42 are myrcene combination products of molecular weight 272 and base peak m/e 69. Subsequent reaction of myrcene under Diels–Alder conditions yielded two main reaction products corresponding in retention time and mass spectral data to these two collected peaks. The experimental findings are thus in agreement with previous

work by Lammens and Verzele (1968), isolating m- and p-camphorene from hop oil, and confirm their explanation of the role of myrcene as the precursor.

While the remaining polymerization components are as yet unidentified, the similarity of their mass spectral cracking patterns to the two identified components would tend to indicate that they are myrcene rather than limonene derivatives.

It is interesting to note that the elevation of the reaction temperature greatly increased polymerization, while its effect upon further oxidation of limonene was by no means as drastic. This would tend to support the proposition that the precursor to most of the combination products volatile to gc is myrcene and not limonene.

Higher polymerization products were experimentally measured during the course of the reactions by distillation of the aliquots at 190° (12 mm) and gravimetric analysis of the residual polymeric tars (Figure 8). Thus, while only 46% of the original myrcene had polymerized in 48 hr at 65°, almost 82% had become nondistillable in 48 hr at 100°. Infrared analysis of the residual gum from these higher polymers showed, instead of the expected trimer and higher hydrocarbon analogs, a resin containing polymeric hydroxyl and ester linkages, quite similar to the resin isolated by Hashimoto (1970) in his work with the degradation of whole hop oil in beer model systems.

SUMMARY

The effects of autoxidation of myrcene have been studied and found to proceed in four reaction classes: cyclization, oxidation, disproportionation, and polymerization. Pathways and intermediates have been proposed and the final products identified. The flavor importance of these final products is the subject of our continuing research and will be reported upon its completion.

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Autoxidation of n-Hexanal. Identification and Flavor Properties of Some Products of Autoxidation

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n-Hexanal was subjected to autoxidation by passing a slow stream of air through the sample at 70° for varying lengths of time. The products of autoxidation were analyzed by gas–liquid chromatographic and thin-layer chromatographic methods. Under the conditions of the experiment, n-hexanal underwent oxidation, polymerization, and degradation, resulting in the production of a number of compounds possessing interesting flavor properties. Some of the flavor notes were similar to those produced in stored cereal grains such as rice, in which n-hexanal is present as an oxidation product of unsaturated fatty acids. In this paper gc, ir, and mass spectral data are presented on the identification of some of the products of autoxidation.

Like other natural products, cereal grains are susceptible to chemical deterioration when subjected to storage at elevated temperatures in the presence of atmospheric oxygen. One of the most important manifestations of such deterioration is the production of off-flavors. A number of carbonyl compounds have been found to be responsible for these off-flavors, and in many instances, lipids, particularly those containing unsaturated fatty acids, have been found to be their major source. Several studies have been reported in the literature on the oxidative deterioration of unsaturated lipids (Brodnitz, 1968), lipid containing products (Watts, 1968), and unsaturated carbonyl compounds (Lillard and Day, 1964), but only a few reports are available on the behavior of saturated fatty acids and saturated carbonyls toward oxidative conditions of storage (Brodnitz, 1968; Watanabe and Sato, 1970). In our laboratories we have found that n-hexanal, an off-flavor compound present in stale rice, undergoes further autoxidation, giving rise to a number of volatile and nonvolatile compounds. In this paper, identification of some products of autoxidation of n-hexanal will be reported.

EXPERIMENTAL SECTION

n-Hexanal was purchased from K&K Laboratories (Plainview, N. Y. 11803), and its purity assayed by gas chromatographic analysis. The sample was found to be 98% pure, the remaining 2% consisting of hexanoic acid. This compound was used for our studies without further purification.

Autoxidation. Fifty milliliters of n-hexanal was placed in a 100-ml round-bottomed flask and subjected to a slow

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