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

S. Rao Palamand* and Robert H. Dieckmann

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

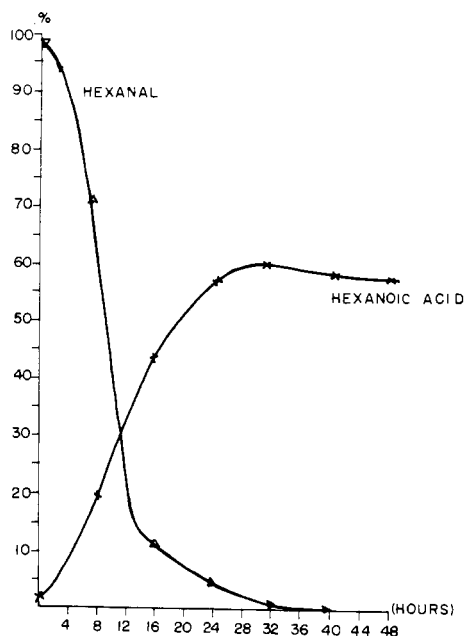Technical Center, Anheuser-Busch, Inc., St. Louis, Missouri 63118.

Figure 1. Rate of autoxidation of hexanal.

Table I. List of Compounds Present in the Sample Represented in Figure 2 (Acid Fraction, Ethyl Esters)

Peak	Compound	Method of ident.
1	Diethyl ether	Gc, ms
2	Acetone	Gc, ms
3	Ethyl acetate	Gc, ms
4	Ethanol	Gc, ms
5	Ethyl butyrate	Gc, ms
6	Ethyl valerate	Gc, ms
7	Ethyl caproate	Gc, ms
8	Ethyl heptanoate	Gc, ms
9	Ethyl caprylate	Gc
10	2,6-Dimethoxyheptanone	Ms
11	Propyl hexyl ketone	Ms
12	Ethyl caprate	Gc, ms
13	Ethyl levulinate	Gc, ms
14	Unidentified	
15	Valeric acid	Gc, ms
16	γ-Hexalactone and an unidentified ester	Gc, ms Ms
17	Ethyl undecanoate	Gc, ms
18	Ethyl dodecanoate and hexanoic acid	Gc, ms Gc, ms
19	Unidentified lactone	Ms
20	Heptanoic acid	Gc, ms
21	Unidentified lactone	Ms
22	Unidentified ester	Ms
23	Ethyl myristate	Gc, ms
24	Caprylic acid and an unidentified ester	Gc, ms Ms
25	Unidentified ester	Ms

stream of air (100 ml/min) for 48 hr at 70°. Two ice water condensers were attached in a series to prevent the loss of reaction products through volatilization. Sample aliquots were removed every 8 hr for analysis by gas chromatography in order to follow the course of the degradation.

Fractionation. Since the chromatogram of the reaction mixture was rather complex, the mixture was separated into acidic and nonacidic fractions, and the two fractions were analyzed by gas chromatography. The acid fraction was esterified before gas chromatographic analysis.

The reaction mixture was dissolved in a minimum of diethyl ether and run through a silica gel (80–100 mesh) column which had been precoated with potassium hydroxide as described previously (Tripp et al., 1968). The neutral fraction was eluted by continuous washing with additional diethyl ether, and the acid fraction was eluted by subsequent washing with 2% phosphoric acid in ether. After removal of the solvent in vacuo, the acid fraction was esterified with ethanol. Esterification was accomplished by refluxing the sample with 15 ml of absolute ethanol in the presence of a catalytic amount of sulfuric acid for 4 hr. The sample was partitioned between diethyl ether and distilled water and the organic layer was rinsed twice more with water to remove residual ethanol and phosphoric acid, dried over magnesium sulfate, and concentrated to near dryness in vacuo. Analysis of both fractions was by gas chromatography and mass spectrometry.

Identification. Identification of the products of autoxidation was attempted by gas chromatographic and mass spectrometric methods.

Gas Chromatography. Gas chromatographic (gc) analysis was carried out using an Infotronics 2420 gas chromatograph equipped with a flame ionization detector and an automatic temperature programmer. Conditions of gc analysis are as listed: column: Teflon, 12 ft × 2 mm (i.d.); packing: 8% FFAP on acid-washed DMCS treated Chromosorb W, 80–100 mesh; injection port temperature: 250°; column temperature: 65–195°, programmed at the rate of 5°/min after holding at 65°/5 min (analysis was terminated after holding at the upper limit for 60 min); detector temperature: 250°; carrier gas: helium, at 40 ml/min; hydrogen: 25 ml/min; air: 200 ml/min; chart speed: 0.5 in./min up to 20 min, then changed to 0.25 in./min.

Mass Spectrometry. All mass spectral data were collected on a Varian MAT 111 GNOM gas chromatographic-mass spectral system equipped with an electron impact ionization detector. The same analytical column

used for gas chromatographic analysis was employed in the gc–mass spectral system, and interfacing was accomplished by the use of a molecular effusion separator. The ionization potential of the ionizing beam was set at 80 eV. Mass spectral scanning was made at the rate of 25 mass units/sec, and several scans of a given peak were obtained in order to determine the homogeneity of the eluting peaks. Spectral identification of compounds was accomplished by reference to published spectra (Cornu and Massot, 1966) in most cases and by interpretation of mass spectral fragmentation patterns in a few cases. The identified compounds were purchased, and the identification data were verified by gas chromatographic peak matching (retention time analysis).

RESULTS AND DISCUSSION

The extent of autoxidation of n-hexanal as a function of time is represented in Figure 1. The figure shows a decrease in concentration of n-hexanal with time when heated at 70° for different lengths of time under a constant flow of air. The figure also shows a plot of concentration of n-hexanoic acid as a function of time, which indicates that the concentration of this acid increases steadily up to 32 hr in the autoxidation process. This graph is presented merely to show that oxidation was taking place in the sample under the conditions of our experiment. Other products of autoxidation are shown in subsequent figures.

Figure 2 shows a gas chromatogram of acidic compounds formed by the autoxidation of n-hexanal at 70° for 48 hr, converted into ethyl esters for the analysis. Identity of the compounds shown in Figure 2 along with the methods used for their identification are listed in Table I. Figure 3 and Table II represent the gas chromatogram and list of compounds identified, respectively, in the nonacidic fractions of the autoxidation products of n-hexanal. These results show that under the conditions of our experiment, n-hexanal undergoes extensive breakdown and participates in a variety of reactions resulting in the formation of a large number of compounds representing several classes.

It is interesting to note that several compounds present in the reaction mixture possess molecular weights much

Table II. List of Compounds Present in the Sample Represented in Figure 3 (Nonacid Fraction)

Peak	Compound	Method of ident.	Peak	Compound	Method of ident.
1	Diethyl ether	Gc, ms	23	Unidentified ester (M + 182)	Ms
2	Acetone	Gc, ms	24	Unidentified lactone	Ms
3	Unidentified ketone	Ms	25	Unidentified ketone	Ms
4	Ethyl acetate	Gc, ms	26	Ethyl laurate and hexanoic acid	Gc, ms
5	Unidentified ketone	Ms	27	trans-Hexenyl caproate	Ms
6	2-Methyl-5-ethylheptane	Ms	28	Unidentified ester	Ms
7	2,2-Dimethylpentane	Ms	29	Unidentified lactone	Ms
8	Hexanal	Gc, ms	30	Unidentified ketone	Ms
9	Unidentified		31	Unidentified ketone	Ms
10	Ethyl valerate	Gc, ms	32	γ-Nonalactone	Gc, ms
11	2-Methylpentanal	Gc, ms	33	δ-Nonalactone	Gc, ms
12	1-Pentanol	Ms	34	γ-Decalactone	Gc, ms
13	δ-Valerolactone	Ms	35	δ-Decalactone	Gc, ms
14	γ-Hexalactone	Gc, ms	36	ε-Decalactone	Gc, ms
15	δ-Hexalactone	Gc, ms	37	Unidentified δ-lactone	Ms
16	Unidentified ketone (M + 128)	Ms	38	Unidentified ester	Ms
17	4-Ethyl-2-octene	Ms	39	γ-Undecalactone	Gc, ms
18	Unidentified ester	Ms	40	δ-Undecalactone	Gc, ms
19	Di-n-amyl ketone	Ms	41	Unidentified δ-lactone	Ms
20	Cyclohexyl propyl ketone	Ms	42	γ-Dodecalactone	Gc, ms
21	2-Dodecenal	Gc, ms	43	δ-Dodecalactone	Gc, ms
22	Unidentified hydroxy ester (M + 154)	Ms			
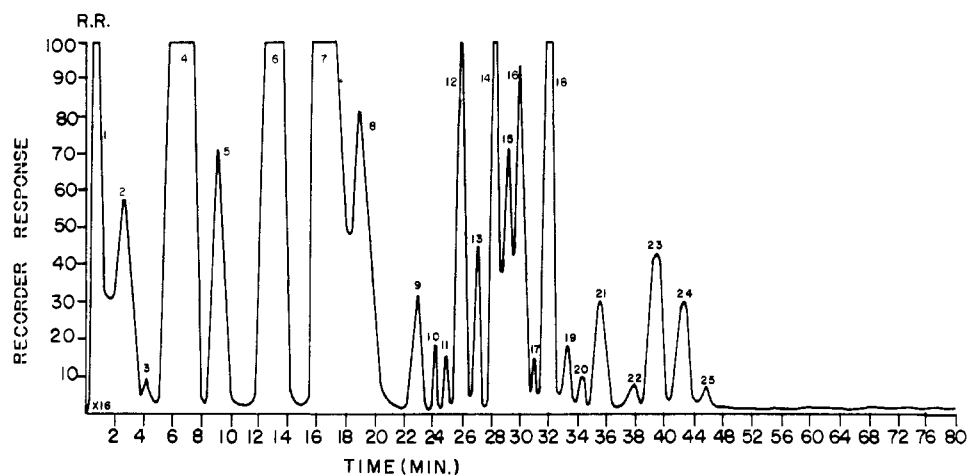
Figure 2. Gas chromatogram of ethyl esters of the acids formed by the autoxidation of hexanal.
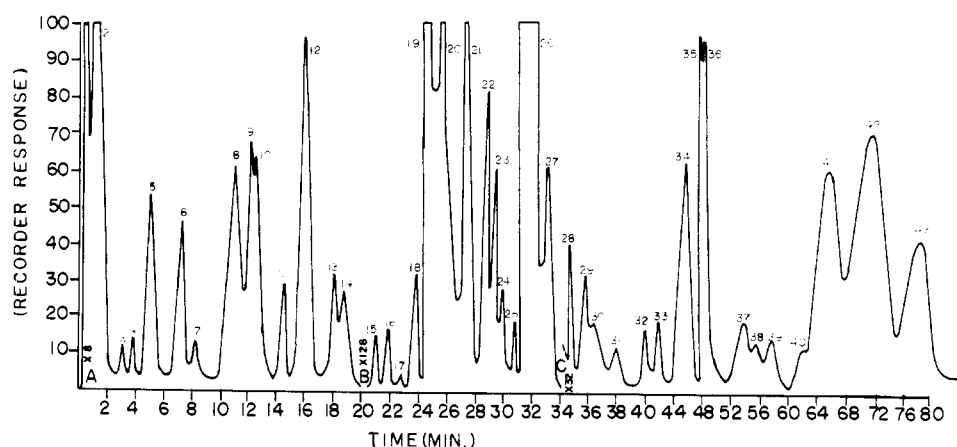
Figure 3. Gas chromatogram of the nonacid fraction of the autoxidation products of hexanal.

higher than that of the parent compound, n-hexanal. This would indicate that breakdown of n-hexanal is followed, in some cases, by recombination of fragments to form the high molecular weight species. The reaction products of our experiments are predominantly esters and lactones (intramolecular esters), carbonyl compounds, and acids,

other compounds such as alcohols and hydrocarbons being present in smaller numbers and lower concentrations.

Brodnitz (1968) and Watanabe and Sato (1970) suggest that autoxidation of saturated fatty acids and aldehydes proceeds via a free-radical mechanism. The presence of hydrocarbons such as methylethylheptane, dimethylpen-

tane, and ethyloctene (peaks 6, 7, and 17, respectively, shown in Table II) support speculation that free-radical reactions could be involved here.

Watanabe and Sato (1970) further suggest that the lactones identified in their work with saturated alcohols, acids, and aldehydes are formed by a free-radical mechanism. These authors used rather drastic conditions for oxidation of the saturated compounds in that they employed 0.1% $KMnO_4$ as the oxidizing agent. In our experiments, comparatively milder conditions were used for autoxidation, but these conditions appear sufficiently adequate to break down almost 100% of the hexanal in about 48 hr.

The small impurity of hexanoic acid present in our sample, along with the large amount of this acid produced by the oxidation of hexanal, would be sufficient to induce acid-catalyzed degradation of hexanal, with subsequent formation of other compounds.

Based on the work reported by Watanabe and Sato (1970), as well as on the nature of compounds formed in our experiments, one is tempted to consider that free-radical reactions were indeed involved in the formation of the numerous compounds, particularly in the formation of various lactones. The lactones formed from the C_{12} aldehyde (Watanabe and Sato, 1970) consisted of C_{12} and smaller carbon chain lengths, whereas, in our studies, we have identified lactones possessing carbon atoms greater in number (peaks 32–43 in Table II) than the parent compound, in addition to the shorter chain lactones (peaks 13, 14, and 15, Table II).

In the formation of lower molecular weight lactones from n-hexanal, it is possible to consider that the first step in this process is the oxidation of n-hexanal to n-hexanoic acid, which could then undergo attack by molecular oxygen at different carbon atoms in the chain as shown in Scheme I. Similarly, δ-hexalactone is formed by the hydroperoxidation of hexanoic acid at the δ-carbon atom. The longer chain compounds (acids, esters, and lactones) identified in this work were probably formed by the addition of alkyl and acyl free radicals in appropriate chain lengths, present in the system, followed by attack on these compounds by molecular oxygen. Also, saturated aldehydes undergo polymerization when subjected to the influence of heat and oxygen and these polymers could conceivably provide further substrates for autoxidation and lactonization reactions.
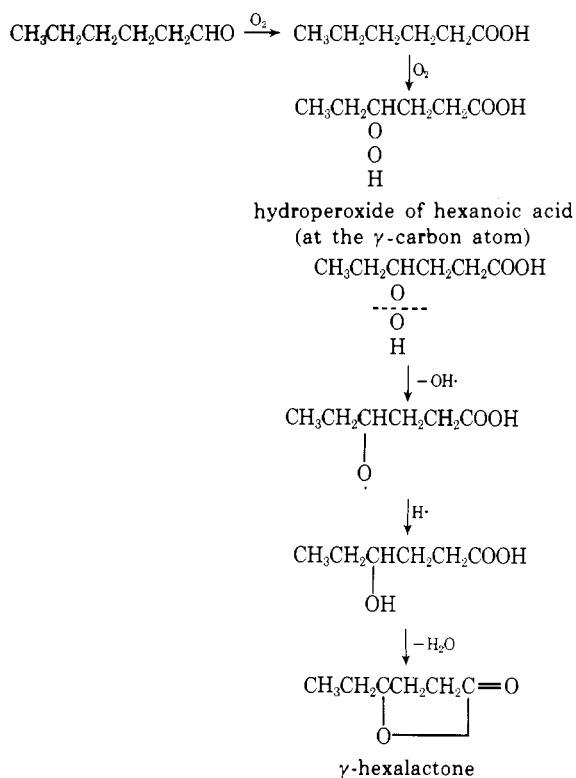
No attempt was made in this study to determine if the autoxidation proceeded with the initial formation of free radicals as this was not our objective. It should be mentioned here that the autoxidation of n-hexanal did indeed produce a great many compounds possessing significant odor and taste properties.

Further work in regard to the identification of the remaining compounds in the reaction mixture of n-hexanal autoxidation as well as the determination of their flavor properties is in progress.

SUMMARY

n-Hexanal was subjected to autoxidation by holding the sample at 70° for 48 hr while passing a stream of air through it.

Scheme I

$$CH_3CH_2CH_2CH_2CH_2CHO \xrightarrow{O_2} CH_3CH_2CH_2CH_2CH_2COOH$$

$\downarrow O_2$

CH$_3$CH$_2$CHCH$_2$CH$_2$COOH
O
O
H

hydroperoxide of hexanoic acid
(at the γ-carbon atom)

CH$_3$CH$_2$CHCH$_2$CH$_2$COOH
O
- - - - -
O
H

$\downarrow$ –OH·

CH$_3$CH$_2$CHCH$_2$CH$_2$COOH
|
O
·

$\downarrow$ H·

CH$_3$CH$_2$CHCH$_2$CH$_2$COOH
|
OH

$\downarrow$ –H$_2$O

CH$_3$CH$_2$CCH$_2$CH$_2$C$=$O
|
O——

γ-hexalactone

The products of autoxidation were separated into acidic and nonacidic compounds by column chromatography and the two fractions were subjected to gas chromatography-mass spectrometry for separation and identification of the various products formed.

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