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Received for review August 23, 1984. Revised manuscript received January 17, 1985. Accepted February 25, 1985.

Inhibition of the Formation of α-*p*-Dimethylstyrene and *p*-Cymen-8-ol in a Carbonated Citral-Containing Beverage System

Val E. Peacock*[1] and David W. Kuneman[1]

The addition of isoascorbic acid to a carbonated beverage system containing 3 ppm citral strongly inhibits the formation of α-*p*-dimethylstyrene (II) and *p*-cymen-8-ol (VI), which contribute to off-flavors in lemon juice. Previous attempts to inhibit this reaction of citral to II and VI with antioxidants have been unsuccessful. Increasing the pH and reducing storage temperature also significantly retard the formation of II and VI. Citric acid degradation of a carbonated beverage containing only citral resulted in the formation of *cis*- and *trans*-*p*-menth-2-ene-1,8-diols, *cis*- and *trans*-*p*-menth-1-ene-3,8-diols, *cis*- and *trans*-*p*-mentha-2,8-dien-1-ols, 2,3-dehydro-1,8-cineole, α-*p*-dimethylstyrene, *p*-cymen-8-ol, *p*-mentha-1,5-dien-8-ol, and *p*-mentha-1(7),2-dien-8-ol but not the disproportionation product α-terpineol as previously reported. In addition, two compounds suspected to be *cis*- and *trans*-isopiperitenols were observed in the above citral beverage. Lithium aluminum hydride reduction of a mixture of isopiperitenone and piperitenone yielded the same two compounds suspected to be the isopiperitenols but no piperitenol.

The extent of the food industry's interest in the acid-catalyzed cyclization of citral, a major component of lemon flavor, in aqueous citric acid is evidenced by the number of independent groups publishing results on this reaction (Clark et al., 1977; McHale et al., 1977; Kimura et al., 1983). This reaction not only reduces the intensity of the lemon flavor in a product due to decreased levels of citral, but also, as observed by Kimura et al., 1983, results in the formation of undesirable off-flavors in the product. Kimura et al., 1983, have blamed α-*p*-dimethylstyrene (II) and *p*-cymen-8-ol (VI), which are oxidation products of the citral cyclization products *p*-mentha-1(7),2-dien-8-ol (VII) and *p*-mentha-1,5-dien-8-ol (V), as being responsible for the undesirable flavor formed in aged lemon juice. Their attempts to inhibit the formation of these undesirable oxidation products by the addition of the antioxidants BHT, BHA, *n*-propyl gallate, α-tocopherol, nordihydroguaiaretic acid, or *n*-tritriacontane-16,18-dione to aqueous citral mixtures proved unsuccessful.

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RESULTS AND DISCUSSION

The mechanism for the transformation of citral to V and VII, and then to II and VI is shown in Figure 1. In this scheme citral is cyclized to the menthadienols V and VII (Clark et al., 1977; McHale et al., 1977; Kimura et al., 1983), and these are then oxidized by what has been reported to be a disproportionation mechanism to the equilibrium mixture of II and VI (Kimura et al., 1983).

Effect of Temperature on the Formation of α-*p*-Dimethylstyrene (II) and *p*-Cymen-8-ol (VI). In Fig-

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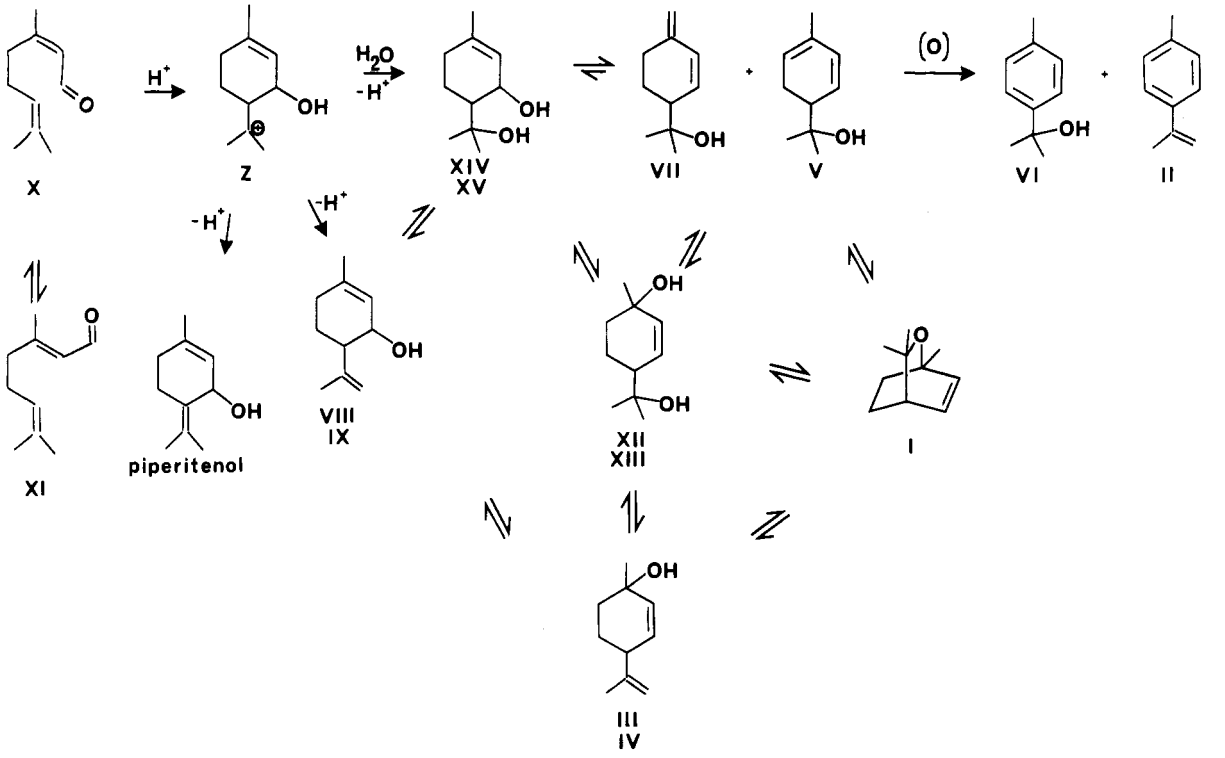

Figure 1. Reaction scheme for the conversion of citral to the other products observed by the citric acid cyclization.

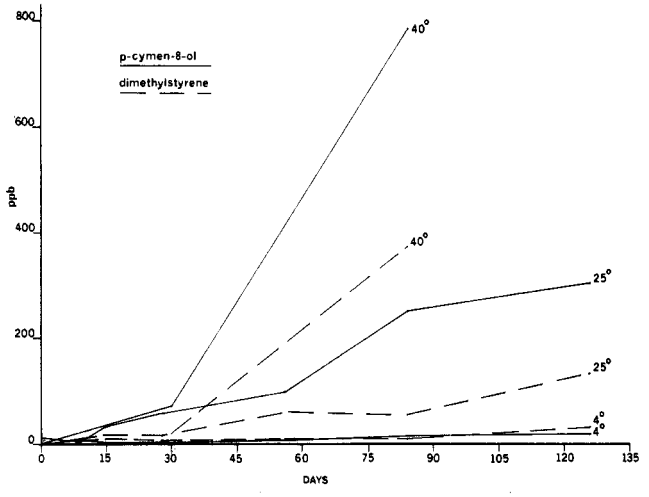

Figure 2. Effect of temperature on the rate of formation of *p*-cymen-8-ol and α-*p*-dimethylstyrene.

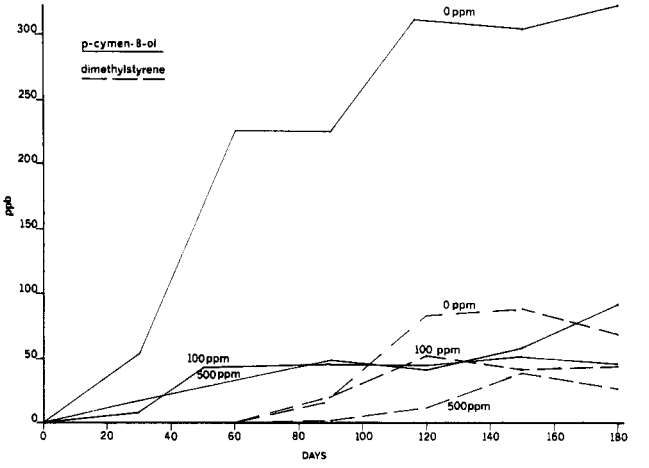

Figure 3. Effect of isoascorbic acid on the rate of formation of *p*-cymen-8-ol and α-*p*-dimethylstyrene.

ure 2 the amounts of II and VI produced in a carbonated citral-containing beverage at pH 3.1 are shown as a function of time and temperature. The rate of transformation of citral to V and VII and then to the equilibrium mixture of II and VI is quite temperature dependent. The amounts of II and VI found in the samples stored at 4 °C remained relatively constant. At 25 °C the rates of formation of these compounds increased moderately, and at 40 °C they increased dramatically. About the same degree of oxidation was observed at 50 days at 40 °C as after 126 days at 25 °C. The rate of appearance of V and VII in this system is highly temperature dependent. This is in part the reason for the acceleration of formation of II and VII at higher temperature. The oxidation step itself is also likely to be accelerated by increasing temperature.

Effect of Isoascorbic Acid on the Formation of II and VI. The traditional method used to control oxidation in a food product is to add an antioxidant. Kimura et al., 1983, observed that the addition of a number of antioxidants to ethanol, 7% citric acid (2:3 v/v) solutions of citral, had not effect on the rate of formation of II and VI either in the presence or absence of oxygen. The effect of isoascorbic acid on the formation of II and VI in a carbonated citral-containing beverage system at 25 °C is presented in Figure 3. After two months, the control contained about 225 ppb VI; however, even after 6 months the samples with 100 ppm and 500 ppm isoascorbic acid contained less than 100 ppb and 50 ppb VI. In addition, after 6 months storage, both antioxidant-containing variables contained only half as much II as the control.

In Figure 4 are shown the isoascorbic acid contents of the test beverages vs. time. The amount of isoascorbic acid in the sample spiked at 100 ppm decreased rapidly during the first month and disappeared entirely after two months. In the sample spiked at 500 ppm, isoascorbic acid decreased rapidly during the first month to a level of 340 ppm and then remained relatively constant for the duration of the study. This would indicate that the isoascorbic acid reacted with some oxidizing agent in the samples (probably dissolved oxygen and oxygen in the headspace of the

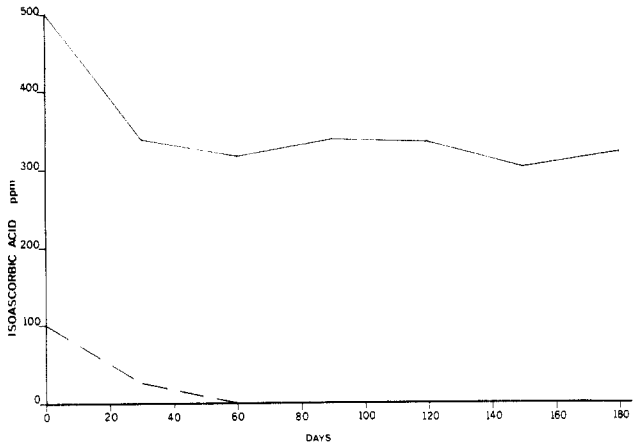

Figure 4. Isoascorbic acid remaining in samples with time.

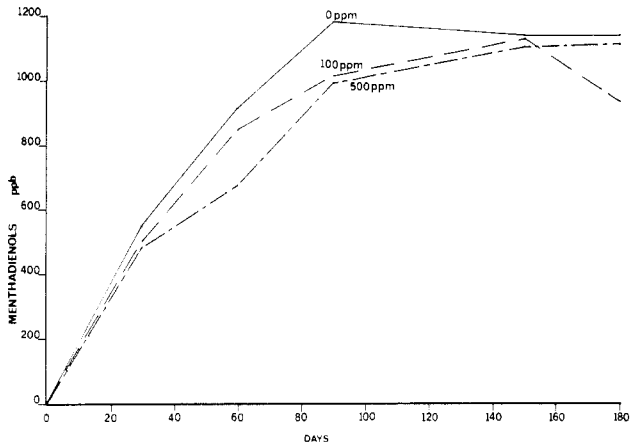

Figure 5. Menthadien-8-ols in samples with time.

bottle) until that oxidizing agent was depleted, after which the remaining antioxidant was stable. As can be seen in Figure 5, there were only minor differences in the amounts of the menthadienols V and VII in the samples with and without added isoascorbic acid. As a result, the massive differences in the rates of formation of II and VI seen in these samples cannot be due to smaller concentrations of the menthadienols V and VII in the samples with added antioxidant, but must be due to inhibition of the oxidation step itself.

Effect of pH on the Formation of II and VI. Another way to reduce the levels of these oxidation products in a carbonated beverage system is to increase the pH of the sample. At pH 3.1 (Figure 6) much more VI and II formed than at pH 3.4 or 3.7. There was much more VI at pH 3.4 than at pH 3.7, but the amount of II in the two samples remained roughly the same with time. This decrease in the rate of oxidation with increased pH is in part the result of the reduced amounts of the citral cyclization products V and VII in the early part of the storage study. For the first 60 days of the storage study, the levels of the menthadienols V and VII observed in the higher pH variables were substantially below those of the pH 3.1 control (Figure 7). Since V and VII form more slowly in the pH 3.7 samples, lower rates of oxidation of these compounds would be expected. However, after about 60 days, when the levels of V and VII are roughly the same at all pHs, the rate of oxidation of these compounds at pH 3.7 should be the same as the other pHs, unless the oxidation reaction itself is pH dependent. The rate of formation of II and VI at pH 3.7 after 60 days still was substantially below that of the samples at lower pHs. This indicates that the oxidation step itself is pH dependent.

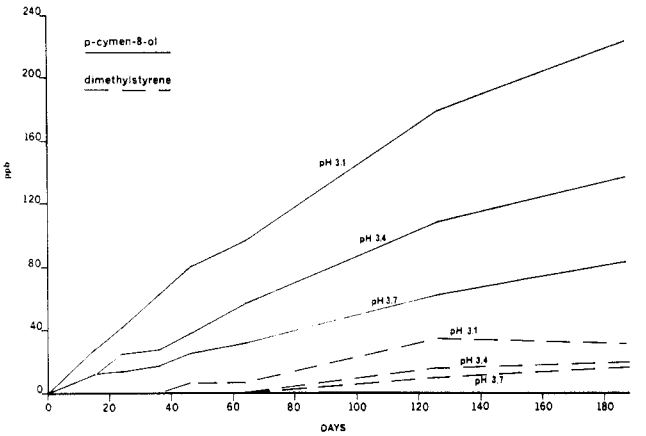

Figure 6. Effect of pH on the rate of formation of *p*-cymen-8-ol and α-*p*-dimethylstyrene at 25 °C.

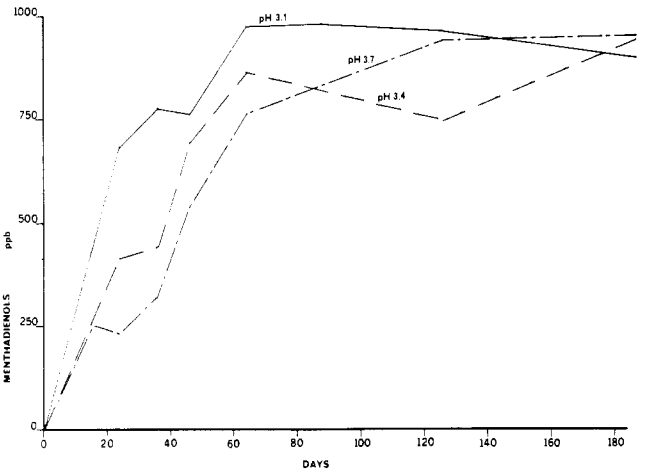

Figure 7. Effect of pH on the rate of formation of *p*-mentha-1,5-dien-8-ol and *p*-mentha-1(7),2-dien-8-ol at 25 °C.

Mechanism of Oxidation. Kimura et al., 1983, have suggested that in their system, this oxidation step proceeds via a disproportionation reaction between menthadienols V and VII to give α-terpineol and VI, which is in equilibrium with II. These authors observed only about 20% as much α-terpineol as *p*-cymen-8-ol (VI) in their model system. This would indicate that this disproportionation reaction might not be responsible for the majority of the *p*-cymen-8-ol produced in their system. As the antioxidants they reported as being ineffective at reducing the rate of oxidation of V and VII are of radical inhibiting nature and not as strong an oxygen scavengers as is isoascorbic acid, perhaps dissolved oxygen in their system may be oxidizing some V and VII. In the present study, when 100 ppm isoascorbic acid was added to samples, after two months all of the antioxidant was destroyed. Yet the rate of formation of II and VI in these samples, after all the antioxidant was depleted, was still strongly reduced vs. that of a sample with no antioxidant added. Also, in the citral only beverage of Table I, none of the disproportionation product α-terpineol was detected. The authors conclude from these observations that under the conditions of the present study, the menthadienols V and VII are reacting with dissolved oxygen in the samples to form VI which is in equilibrium with II, and that very little disproportionation is taking place.

The oxidation potential of oxygen in water is known to increase with decreasing pH (Diehl, 1970). This would explain the pH dependence of the oxidation step.

Isoascorbic acid was chosen over ascorbic acid in this study for commercial reasons. The two isomers are known

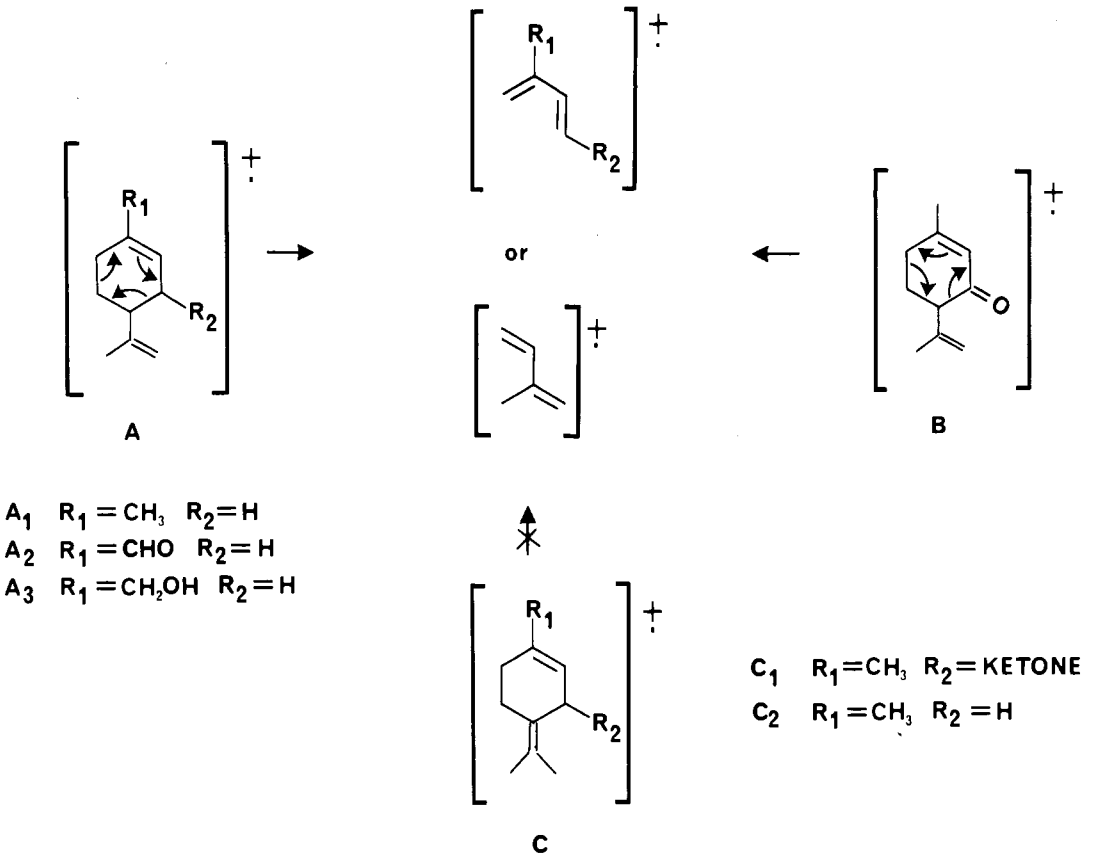

Figure 8. Retro-Diels–Alder fragmentation of the radical cations of terpene cyclohexenes.

to have similar antioxidative properties. Our more limited experience with the addition of ascorbic acid to similar test mixtures showed qualitatively the same results.

Detection of *cis*- and *trans*-Isopiperitenols (VIII and IX) and *cis*- and *trans*-*p*-Mentha-2,8-dien-1-ols (III and IV). The report by Baines et al., 1970, that piperitenol and isopiperitenol (VIII and IX) are the major *initial* cyclization products of citral at pH 1.8 (comprising 37.8% of the reaction mixture after 4 h) has been severely questioned by more recent investigators (Clark et al., 1977; McHale et al., 1977; Kimura et al., 1983). These investigators have unanimously concluded that *p*-mentha-1,5-dien-8-ol (V), *p*-mentha-1(7),2-dien-8-ol (VII), and under some conditions, the *p*-mentha-2-ene-1,8-diols (XII and XIII) are the major reaction intermediates arising from the acid-catalyzed cyclization of citral. None of these authors have detected either piperitenol or isopiperitenol. The general consensus of these more recent works is that isopiperitenol and piperitenol, if present, might be transient intermediates initially formed from citral which, due to their inherent instability in acidic media, isomerized to the other products in the reaction mixture too quickly to be detected.

The change in composition with time of a carbonated beverage system containing only citral (4 ppm) and sucrose (10%) in a citric acid–sodium citrate buffer at pH 3.15 is presented in Table I. The citral isomers neral (X) and geranial (XI) exhibit a half-life of about 6 days under these conditions. Of the reaction products listed in Table I, all but the isopiperitenols (VIII and IX) and the *p*-mentha-2,8-dien-1-ols (III and IV) have been well-characterized by Clark et al., 1977, or McHale et al., 1977. Gas chromatographic–mass spectral data for all these compounds agreed well with the literature. The mass spectrum and retention index on a methylsilicone GC column observed for *cis*-*p*-mentha-2,8-dien-1-ol (IV) agreed well with that reported by Swigar and Silverstein, 1981. The mass spectrum observed for *trans*-*p*-mentha-2,8-dien-1-ol (III) closely agreed with that reported by Noever de Brauw et al., 1979.

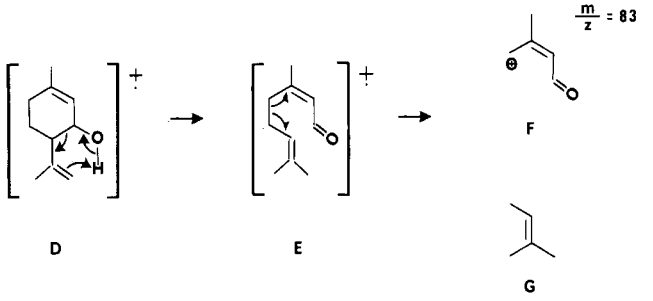

Figure 9. γ-Hydrogen rearrangement of the radical cation of isopiperitenol.

Reference mass spectral and GC retention data for the isopiperitenols were unavailable. The mass spectra of the two isomers suspected to be the isopiperitenols are indistinguishable. Both exhibit a molecular ion at m/z 152 with the only major peaks appearing at m/z 83 and 84.

The major fragmentation pathway for the radical cations of terpene cyclohexenes such as A in Figure 8 is via a retro-Diels–Alder type reaction (McLafferty, 1967). This results in mass spectra wherein the major peaks for the compound correspond to the radical cations of the dienes produced. In the case of the isopiperitenols, this would be m/z 84. The radical cations of compounds such as limonene, A_1, perillaldehyde, A_2, perillyl alcohol, A_3 (Figure 8A), and isopiperitenone (Figure 8B) all undergo this type of fragmentation to produce the most abundant peak of their mass spectra. The radical cations of the cyclohexene terpenes with a 4,8-double bond such as piperitenone C_1 and terpinolene C_2 (Figure 8C) normally do not decompose by a retro-Diels–Alder fragmentation. Thus it would seem unlikely that piperitenol would yield a m/z 84 peak as the most abuntant peak in its mass spectrum.

Table I. Citric Acid Degradation of Citral at pH 3.15 with Time

no.	compd	concn, ppb							retention index
		1 day	5 days	8 days	13 days	28 days	42 days	69 days	
I	2,3-dehydro-1,8-cineole	16	24	57	42	61	68	71	985
II	α-*p*-dimethylstyrene		13	15	5	9	9	16	1080
III	*trans*-*p*-mentha-2,8-dien-1-ol	4	18	28	29	33	24	15	1111
IV	*cis*-*p*-mentha-2,8-dien-1-ol	4	17	25	25	31	22	14	1125
V	*p*-mentha-1,5-dien-8-ol	9	73	128	214	379	417	430	1156
VI	*p*-cymen-8-ol		5	14	27	103	162	277	1169
VII	*p*-mentha-1(7),2-dien-8-ol	11	72	130	194	288	315	346	1181
VIII	isopiperitenol A	11	27	40	41	46	36	18	1192
IX	isopiperitenol B	3	5	13	9	12	8	7	1210
X	neral	1317	838	601	305	57	13		1225
XI	geranial	2312	1441	1023	525	98	27		1252
XII	*trans*-*p*-menth-2-ene-1,8-diol	181	699	993	1158	1426	1375	1284	1268
XIII	*cis*-*p*-menth-2-ene-1,8-diol	136	545	798	932	1141	1068	1022	1285
XIV	*trans*-*p*-menth-1-ene-3,8-diol	2	31	63	51	70	72	41	1340
XV	*cis*-*p*-menth-1-ene-3,8-diol	4	37	69	72	102	99	40	1362

The m/z 83 peak for the isopiperitenols can be explained by a γ-hydrogen rearrangement (McLafferty, 1967) as illustrated in Figure 9, followed by cleavage of the doubly allylic carbon–carbon bond, to give the delocalized cation F (m/z 83) and the allylic radical G. The radical cations of compounds V, VII, XII, and XIII all decompose by what appears to be this type of γ-hydrogen rearrangement, from which the most abundant peak of all of their mass spectra (m/z 94) results.

In order to provide reference samples of the isopiperitenols, an attempt was made to synthesize these compounds by the lithium aluminum hydride reduction of a mixture of 75% piperitenone and 25% isopiperitenone. As has been reported for the reduction of piperitenone with aluminum isopropoxide (Jones and Webb, 1972), what was observed in the reaction mixture was predominately a mixture of *p*-menthatriene isomers. However, about 23% of the crude reaction mixture consisted of two compounds which had mass spectra and GC retention times identical with those of the two compounds believed to be the isomeric isopiperitenols from the citral degradation mixture. An attempt to isolate these compounds by column chromatography on alumina was unsuccessful. As a result it was not possible to determine which of the observed isomers was *cis*-isopiperitenol and which was *trans*-isopiperitenol.

In view of the observation of the same compounds in the lithium aluminum hydride reduction of isopiperitenone and piperitenone (which should result in the corresponding alcohols) as was found in the citral degradation mixture, it would seem improbable that these compounds are anything other than the isopiperitenols. Since piperitenol is unlikely to have a m/z 84 peak as the most abundant peak of its mass spectrum, it is improbable that either of these compounds is piperitenol. These isopiperitenols are also observed by the extraction of aged lemon beverages by ether and by the method of trapping flavor volatiles on a C-18 Sep-Pak (Waters Associates) as described in the Experimental Section. As a result, these isopiperitenols are unlikely to be artifacts of the chloroform extraction.

In Figure 1, a scheme is proposed for the transformation of citral to all the products observed in the present work. This scheme differs from previously reported ones in that here the carbonium ion Z can not only hydrate to the 3,8-diols, XIV and XV, but can deprotonate to the isopiperitenols, VIII and IX. In addition, piperitenol might be formed also by deprotonation; but as none was detected, it must either quickly decompose or is not formed competitively with the other products.

The *p*-mentha-2,8-dien-1-ols (III and IV) have not been previously reported in degradation mixtures of citral. Their formation would not be unexpected in the presence of the isopiperitenols (VIII and IX) since piperitol is known to equilibrate with *p*-menth-2-en-1-ol under acidic conditions (Bain et al., 1959).

EXPERIMENTAL SECTION

Experimental Samples. The reaction mixtures of Figures 2–7 were prepared with a natural lemon flavor yielding a final citral concentration of ca. 3 ppm. All samples contained 10% sucrose (w/v) and 0.12% citric acid; the pH was adjusted with sodium citrate. The products of Figure 2 were packed in 16-oz. green-glass, twist-cap bottles. The other samples were packed in 10-oz. green-glass, crown-cap bottles. All samples were carbonated to 3.8 volumes of CO_2 and stored in the dark.

The carbonated beverage of Table I was made identically to the above samples, only substituting citral (Aldrich Chem. Co.) for the lemon flavor. Green-glass, crown-cap bottles (300 mL) were filled with this product, crown-capped, and stored in the dark at 25 °C. The initial product had a pH of 3.15 and contained about 4 ppm citral.

Analytical Methods. The variables of Figure 2 were analyzed by the following method: The sample (1500 mL, degassed) was extracted with ether (2 × 300 mL). The combined ether fractions were dried with anhydrous $MgSO_4$, filtered, and concentrated to a volume of about 5 mL with a six-chamber (noninsulated) bubble column at ambient pressure. This concentrate was spiked with a known amount of jasmone as an internal standard. Flavor volatiles were quantified by GC method A below. The detection limit of this technique was roughly 10 ppb with a reproducibility of ±10%.

The variables of Figures 3, 5, 6, and 7 were analyzed by the following method: Methanol (5 mL) was drawn through a C-18 Sep-Pak (Waters Associates) with a vacuum aspirator (100 mmHg). Water (2 mL) was aspirated through the Sep-Pak followed by 200 mL of the sample. With the use of a syringe, methanol (2 mL) then was forced through the Sep-Pak to elute the adsorbed flavor volatiles. A known amount of jasmone then was added to the sample as an internal standard. Flavor volatiles were quantified by GC method B. The detection limit and reproducibility of this technique were similar to those of the first method.

The citral beverage of Table I was analyzed by the following method: Three 300-mL bottles of the sample were combined and extracted with 350 mL of chloroform in a 1-L continuous liquid–liquid extractor. A 100-mL side boiling flask was used. The extraction was continued for 24 h. The chloroform from the side flask and the remaining chloroform from the extractor were combined, dried with anhydrous Na_2SO_4, and concentrated to a

volume of 5–10 mL by distillation with a noninsulated six-chamber bubble column. A solution of 1% jasmone (Pfaltz and Bauer Inc.) (10 μL) in methanol was added as an internal standard. This solution was injected directly into the GC for analysis by method C below.

Gas Chromatographic Methods. Method A: A 50 m × 0.25 mm i.d. Hewlett-Packard OV-101 column was used with nitrogen as the carrier gas. A Hewlett-Parkard 5880 GC equipped with a capillary injector and an FID detector, temperature programmed at 45 °C for 1 min, then to 250 °C at 4 °C/min, was used. As in the other methods a 1-μL sample was injected with a 2:1 split ratio.

Method B: The above column and temperature program were used with a Hewlett-Packard 5985B GC/mass spectrometer with a capillary injector and with He as the carrier gas. Quantification was done by using total ion current mass spectrometry for all the compounds except VI. This compound was quantified by select ion monotoring at m/z 135. The response for VI (VI coeluted with terpinen-4-ol which shows no m/z 135 peak) at m/z 135 was 11.2 times that of an equal amount of jasmone under the conditions used.

Method C: A 50 m × 0.25 mm i.d. bonded SE-30 column (Quadrex Corp.), a Hewlett-Packard 5840 GC equipped with an FID detector, and an 18835C capillary inlet system were used. The above temperature program was used. Retention indices for the reported compounds were determined with the same GC conditions except using a 100-m column. The chloroform concentrates were spiked with methyl hexanoate, methyl octanoate, methyl decanoate, and methyl dodecanoate. When the retention indices reported by Jennings and Shibamoto, 1980, for these standards were used, the retention indices of Table I were calculated.

A Hewlett-Packard 5985B GC/mass spectrometer, under the above conditions was used to obtain mass spectral data.

Isopiperitenol (A) (VIII). MS, m/z 152 (4), 137 (3), 119 (9), 108 (10), 91 (9), 84 (100), 83 (49), 81 (9), 56 (8).

Isopiperitenol (B) (IX). MS, m/z 152 (4), 137 (4), 134 (4), 121 (8), 119 (5), 109 (9), 108 (10), 91 (9), 84 (100), 83 (50), 79 (6), 77 (6), 69 (9), 56 (8).

Reduction of Piperitenone and Isopiperitenone. Lithium aluminum hydride (200 mg, 0.006 mol) was added to 80 mL of dry ether. Added dropwise to this stirred slurry was 0.65 g (0.0043 moles) of a 75:25 mixture of piperitenone and isopiperitenone (SCM Organic Chemicals) dissolved in 10 mL of ether. After the addition was complete, the reaction was stirred 4 h. The reaction was quenched by the dropwise addition of 0.2 mL of water, followed by 0.2 mL of 15% aqueous NaOH and then 0.6 mL of water. The reaction was stirred until the lithium salts precepitated completely (about 16 h) and then filtered. This crude ether solution was used directly for GC/MS analysis. The reaction products were mostly menthatrienes from the piperitenone (Jones and Webb, 1972) and about 23% isopiperitenols. The isopiperitenols could not be recovered after being applied to an alumina column in an attempt to isolate pure samples of these compounds.

Isoascorbic Acid Assay. Ten microliters of sample was degassed and immediately 0.5 mL of 20% H_3PO_3 was added. A 300 ppm reference sample was prepared by addition of 30 mg of isoascorbic acid to 100 mL of water followed immediately by 5 mL of 20% H_3PO_3. A 10-μL sample was injected onto a Waters Associates amino column in a Z module. The mobile phase was 15% acetonitrile/0.05 M H_3PO_3, adjusted to pH 6.0 with 20% KOH. A flow rate of 1.0 mL/min was delivered by using a Waters Associates M-45 pump. Peaks were quantified by UV adsorption at 280 nm.

ACKNOWLEDGMENT

We thank J. Campbell and P. McCamant of this department for the manufacture of the experimental samples, M. Grace of this department for determination of retention indices, and SCM Organic Chemicals of Jacksonville, FA for donation of the piperitenone–isopiperitenone mixture.

Registry No. I, 66113-06-2; II, 1195-32-0; III, 7212-40-0; IV, 3886-78-0; V, 1686-20-0; VI, 1197-01-9; VII, 65293-09-6; X, 106-26-3; XI, 141-27-5; XII, 54164-90-8; XIII, 54164-91-9; XIV, 95723-03-8; XV, 87859-09-4; isoascorbic acid, 89-65-6; citral, 5392-40-5; citric acid, 77-92-9; *cis*-isopiperitenol, 4017-76-9; *trans*-isopiperitenol, 4017-77-0.

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Received for review July 2, 1984. Revised manuscript received November 26, 1984. Accepted February 4, 1985.