Note

Relationship between Acid-catalyzed Cyclization of Citral and Deterioration of Lemon Flavor[†]

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Previously,^{1~5)} we have reported that *p*-cymene and 2-*p*-tolyl-propene among the degradation products of citral are significant constituents responsible for the off-odor of deteriorated lemon. Citral consists of a mixture of geranial (1, *E* isomer) and neral (2, *Z* isomer) in a ratio of about 65 to 35, respectively. So far, studies on the deterioration of lemon flavor has been performed using a citral mixture since it was not easy to isolate each geometrical isomer on a large scale.

In this paper, to elucidate a deterioration mechanism of lemon flavor, citral was separated to geranial and neral by

high performance liquid chromatography (HPLC) in advance and each pure compound was treated with a 5% citric acid aqueous solution. At intervals during incubation, the deterioration products in each *n*-hexane extract were determined using gas chromatography (GC) and mass chromatography (MC).

The GC conditions were the same as a previous paper,⁵⁾ except for choosing a column temperature of 100°C to 160°C at a program rate of 5°C/min. Mass spectra were measured on a JEOL JMS-D300 spectrometer. GC-MS conditions were as follows: column, 2 mm i.d. × 1 m glass column packed with 5% DEGS on Uniport HPS (80~100 mesh); column temperature, programmed from 60°C to 200°C at 3°C/min; carrier gas flow rate (N₂), 25 ml/min; ionizing voltage, 24 eV; accelerating voltage, 3 kV; ion source temperature, 200°C. MC was carried out using a JEOL JMA-2000 Mass Data Analysis System. GC-MS conditions are the same as those mentioned above. HPLC was performed on System 500 Water Associates apparatus with a Prep-500 Silica column (30 cm × 57 mm i.d. × 2). The solvent was isopropyl ether-*n*-hexane (10:90, v/v) and the flow rate was 300 ml/min. IR spectrum was taken on a Hitachi-285 grating infrared spectrometer. NMR spectrum was measured on a JEOL-FX 200 FT spectrometer (200 MHz) with (CH₃)₄Si as an internal standard.

Citral (a mixture of neral and geranial) was purchased from Tokyo Kasei Co. Neral and geranial were separated from the citral by HPLC. Each chemical structure was confirmed by spectral data.

The important intermediate, *p*-mentha-1,3-dien-8-ol (4) was isolated and identified according to the following procedure. Citral (1 g, 6.58 mmol) dissolved in 30 ml of ethanol was put into 160 ml of 5% citric acid aqueous

FIG. 1. Fate of Geranial and Formation of Intermediates in Citric Acid Aqueous Solutions.

●—●, geranial; ○—○, neral; ▲—▲, intermediate (*p*-mentha-1,3-dien-8-ol); △—△, intermediate (an isomer of *p*-mentha-1,3-dien-8-ol); ×—×, 2-*p*-tolyl-2-propanol; □—□, *p*-cymene; ■—■, 2-*p*-tolyl-propene.

[†] Studies on the Deterioration Mechanism of Lemon Flavor. Part I.

SCHEME I. Plausible Deterioration Mechanism of Citral in Acid Aqueous Solution.
Compounds in brackets are not completely identified yet.

solution. The mixture was incubated at 38°C for 24 hr with shaking. The deteriorated citral was extracted with three 70 ml portions of *n*-hexane. After washing with brine and drying over anhydrous sodium sulfate, the deterioration products were chromatographed over a neutral aluminum oxide (activity III) column using an *n*-hexane-ether gradient eluate. The *n*-hexane-ether (5:5, v/v) eluate was rechromatographed over aluminum oxide and 10% AgNO₃-SiO₂ columns. From the *n*-hexane-ether (7:3, v/v) eluate, the intermediate (4) was isolated: EI-MS *m/z* (%): no M⁺ detected, 137 (M⁺ - CH₃, 0.5), 134 (M⁺ - H₂O, 0.4), 94 (85), 79 (59), 59 (100). FI-MS *m/z* (%): 152 (M⁺, 100), 135 (M⁺ - OH, 15), 94 (42), 93 (35), 59 (83). IR $\nu_{\max}^{\text{film}}$ cm⁻¹: 3380 (OH), 1640 (C=C), 1440 and 1370 (isopropyl). ¹H-NMR $\delta_{\text{TMS}}^{\text{CDCl}_3}$: 1.18 (3H, s, CH₃), 1.23 (3H, s, CH₃), 1.57 (1H, s, variable OH), 1.69 (3H, s, C=C-CH₃), 5.91 (1H, d, *J* = 10 Hz, -C=CH-), 6.24 (1H, d, *J* = 10 Hz, -C=CH-).

To elucidate a deterioration mechanism for citral, an ethanol-citric acid aqueous solution of citral was incubated at 38°C for 1, 3, 5, 7, 15, 24, 48, 96, 144 and 192 hr. At intervals during incubation, the deteriorated citral was analyzed by GC, GC-MS and MC. Geranial (1) and neral (2) were individually treated by the same method for citral mentioned above.

Figure 1 shows the fate of geranial and the formation of intermediates in the citric acid aqueous solutions. At the first stage, geranial rapidly decreased and neral produced by isomerization of geranial increased in one hour. The equilibrium between the *E/Z* isomers reached a ratio of 65 to 35, which was quantitatively determined by mass chromatography using a JEOL JMA-2000 Mass Data Analysis System. Both terpene aldehydes decreased with the equilibrium ratio after 3 or 5 hr. On the other hand, intermediates such as monoalcohol increased up to 24 hr. Each molecular ion peak was *m/z* 152, C₁₀H₁₆O and one of them was identified as *p*-mentha-1,3-dien-8-ol by IR, MS and ¹H-NMR, and others were isomers of it. They

began to decrease by 48 hr. After that, chemically stable *p*-cymene and 2-*p*-tolyl-propene, which are major components responsible for the off-odor of deteriorated lemon, were formed. In the case of neral, isomerization to geranial and cyclization to the *p*-menthane derivatives competitively occurred at the first stage. After reaching an equilibrium ratio of the *E/Z* isomers, the same deterioration process as the case of geranial was observed.

A plausible deterioration mechanism of citral in the acid aqueous solutions was proposed as shown in Scheme I. The cyclic compounds are directly produced from neral rather than from geranial. Many biosynthetic investigations concerning isomerization of geranyl pyrophosphate and neryl pyrophosphate have been performed.⁶⁻⁸ It seems that the biosynthetic and chemical *cis-trans* isomerizations are very similar. Neral is first cyclized to 1-*p*-menthen-3,8-diol (3)⁹ which is an extremely unstable compound in acid aqueous solution. The unisolable compound (3) is assumed to be immediately transformed to intermediates (4, 5 and 6) by dehydration reactions. Furthermore, those intermediates, which must be unstable under acidic conditions, are changed to give more stable aromatic compounds (7, 8 and 9) by oxidation and/or dehydration reactions. *p*-Cymene (8) and 2-*p*-tolyl-propene (9) are responsible for the significant off-odor of deteriorated lemon.

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