

Volatile Flavor Components of Caucas

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This is the first paper on caucas flavor, which was analyzed using gas-liquid chromatography and mass spectrometry, and the volatile flavor components were identified by direct comparison with the authentic compounds. Many of the components were aliphatic sulfur-containing compounds: methyl allyl sulfide; dimethyl disulfide; diallyl sulfide; methyl *n*-propyl disulfide; methyl allyl disulfide; dimethyl trisulfide; *n*-propyl allyl disulfide; diallyl

disulfide; and methyl allyl trisulfide. 2-Methyl-2-pentenal, probably one of the breakdown products of 1-propenylsulfenic acid (lachrymatory substance), was found in caucas. Methyl 1-propenyl disulfide and allyl 1-propenyl disulfide or di-1-propenyl disulfide were tentatively identified. The main volatile components attributed to the caucas flavor were methyl allyl disulfide, diallyl disulfide, and methyl allyl trisulfide.

In recent years the compositions of the vapor of chopped *Allium* tissue have been much investigated by using the gas chromatograph and the mass spectrometer. The characteristic flavor of onion (*A. cepa* L.), garlic (*A. sativum* L.), and other *Allium* species has been shown to be developed by an enzymatic process and the flavor precursors were shown to be sulfoxide amino acids (Stoll and Seebeck, 1951; Virtanen and Matikkala, 1959) such as *S*-alkyl-L-cysteine sulfoxide (alkyl:*n*-propyl; 1-propenyl; methyl; allyl). Wahlroos and Virtanen (1965) and Bernhard (1968) proposed that the pungent and stimulating flavor of onion was mainly attributable to dipropyl disulfide. Similarly it has been made clear that dimethyl disulfide has a strong cabbage-like odor and that diallyl disulfide, produced as one of the breakdown products of allicin (diallyl thiosulfinate), has a garlic-like odor.

Recently, Brodnitz *et al.* (1969) identified methyl *cis*-1-propenyl disulfide, methyl *trans*-1-propenyl disulfide, *cis*-1-propenyl propyl disulfide, *trans*-1-propenyl propyl disulfide, two methyl 1-propenyl trisulfides, and two 1-propenyl propyl trisulfides as the important components of onion oil.

The authors (Nishimura *et al.*, 1970, 1971) have investigated the volatile flavor products from γ -irradiated solutions of characteristic sulfoxide amino acids in the *Allium* species. It was of interest to investigate the volatile flavor components of caucas (*A. victorialis* L.) in terms of enzymatic reactions with sulfoxide amino acid in *Allium* species and in terms of the chemotaxonomy. The leaves of the caucas, which belongs to the *Allium* species, are nutritious vegetables. Samples were taken from the pickled variety.

Yurugi *et al.* (1954) detected *S*-alkylmercapto-L-cysteine (alkyl:allyl; methyl; propyl) from the reaction between thiamine and ingredients of *Allium victorialis* L. in studies on vitamin B₁.

This paper deals with the isolation and characterization of the volatile flavor components of caucas which have not yet been reported. The components were analyzed using gas-liquid chromatography and mass spectrometry. Moreover, the structure of these components was identified by direct comparison with the authentic compounds.

EXPERIMENTAL

Sample Separation. About 10 kg of caucas were collected at the Nopporo primeval forest (Hokkaido) in May. About 400 g of caucas were cut into small pieces and were placed in a three-necked flask. Volatile flavor components were distilled off by passing through nitrogen gas as a carrier at approximately 40° C, and absorbed into about 50 ml of isopentane trap cooled with Dry Ice-ethanol.

Gas Chromatography. Components of the isopentane extract were fractionated with a Yanagimoto GCG 550 (FID) gas chromatograph on a 1.5-m \times 5-mm i.d. stainless steel column packed with 20% Reoplex 400 on 60 to 80 mesh acid-washed C-22 (firebrick) at a helium flow rate of 30 ml/min. The temperature was 120° C (injection port temperature, 180° C). Moreover the gas chromatogram of the isopentane extract was compared with that of the headspace vapor. Exactly 300 g of homogenized caucas was placed in a 500-ml silicone-capped glass vessel. Volatile samples (4.0 ml of vapor) for gas chromatographic analysis were obtained from headspace of the vessel after standing at 50° C for 5 min. To obtain the flavor components fractionated, a FID gas chromatograph in which a splitter was inserted was also used under the same condition. Each fraction was collected in a glass tube cooled with Dry Ice-ethanol.

Mass Spectrometry. To identify the purified components, a Hitachi Model RMU-6E mass spectrometer was used. The operating parameters were as follows: inlet temperature, 200° C; ion source pressure, 3×10^{-7} mm; ion source temperature, 250° C; acceleration voltage, 1.8 kV; chamber voltage, 80 eV.

Gas Chromatography-Mass Spectrometry (gc-ms). The method of gc-ms combination was preferred to obtain the mass spectral data of trace amounts of components. A Watson-Biemann (Watson and Biemann, 1965) helium separator was used between the gas chromatograph (Hitachi Model K-53 gas chromatograph) and the mass spectrometer (Hitachi Model RMS-4 mass spectrometer). The operating parameters were as follows. Gas chromatograph: column, 2-m \times 3-mm i.d. stainless steel column packed with 20% Reoplex 400 on 60 to 80 mesh acid-washed C-22; flow rate, helium carrier gas at 30 ml/min; temperature, 120° C; injection port temperature, 180° C. Mass spectrometer: inlet temperature, 150° C; ion source pressure, 7×10^{-6} mm;

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Table I. Identification of Volatile Components Found in Caucas

Peak no.	Compound	Identification
2	Methyl allyl sulfide	Ms, ^a Rt, A
4	M ⁺ 94	Ms, T
6	Dimethyl disulfide	Ms, Rt, A
7	Methyl <i>n</i> -propyl ether	Ms, T
9	Diallyl sulfide	Ms, Rt, A
10	2-Methyl-2-pentenal	Ms, Rt, A
13	Methyl <i>n</i> -propyl disulfide	Ms, Rt, A
14	Methyl 1-propenyl disulfide	Ms, T
15	Methyl allyl disulfide	Ms, Rt, A
16	Dimethyl trisulfide	Ms, Rt, A
18	<i>n</i> -Propyl allyl disulfide	Ms, Rt, A
19	1-Propenyl-containing disulfide	Ms, T
20	Diallyl disulfide	Ms, Rt, A
21	Methyl allyl trisulfide	Ms, Rt, A

^a Ms = mass spectrometry; Rt = gas chromatographic retention time; A = authentic compound; T = tentative.

ion source temperature, 200° C; target current, 60 μA; total emission, 80 μA; chamber voltage, 70 eV.

Reference Compounds. The following compounds were synthesized by a modification of the method of Kirner and Richter (1929): methyl allyl sulfide; dimethyl sulfide; diallyl sulfide; methyl *n*-propyl disulfide; methyl allyl disulfide; dimethyl trisulfide; *n*-propyl allyl disulfide; diallyl disulfide; and methyl allyl trisulfide. 2-Methyl-2-pentenal was prepared by the method of Paquin (1949).

These compounds were purified by using the gas chromatograph and were confirmed by mass spectrometry and elemental analysis.

RESULTS AND DISCUSSION

The gas chromatographic separation of volatile flavor components of caucas is shown in Figure 1.

The volatile components were identified by comparing with mass spectra and gas chromatographic retention time of the reference compounds. The components identified are presented in Table I. The peak numbers refer to those of Figure 1. The volatiles of caucas contain strong Chinese chive-like (*A. tuberosum*) and garlic-like odors, a weak pickle-like odor, and a very weak lachrymatory factor.

In the sensory test of each fraction eluted from the gas chromatograph, the peaks 15, 20, and 21 gave the characteristic Chinese chive-like odor, garlic-like odor, and pickle-like odor, respectively. Therefore the caucas flavor is assumed to be attributable to the mixture of methyl allyl disulfide (peak 15), diallyl disulfide (peak 20), and methyl allyl trisulfide (peak 21).

The gas chromatogram pattern of the headspace vapor from caucas is somewhat similar to that from garlic. But, compared with the relative peak area of methyl allyl disulfide from caucas and garlic, the former seems to be considerably more than the latter.

2-Methyl-2-pentenal (peak 10), probably produced as one of the breakdown products of 1-propenyl sulfenic acid which has been regarded as a lachrymatory substance (Bredenberg *et al.*, 1962), was found even in caucas. [Brodnitz and Pascale (1971) have recently reported that the lachrymatory factor was isolated and proved to be thiopropanal *S*-oxide.] The mass spectrum of the peak 10 (Figure 1) is shown in Figure 2.

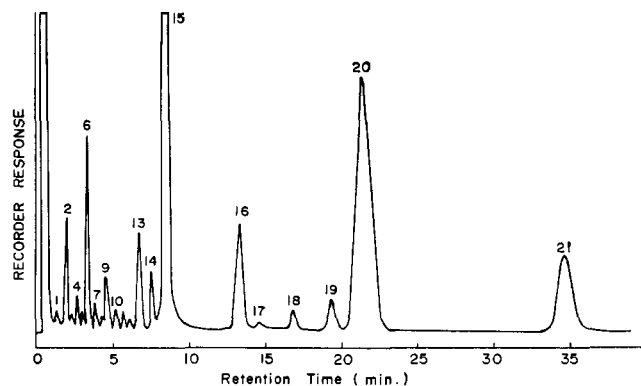


Figure 1. Gas chromatogram of the volatile components of *Allium victorialis* L.

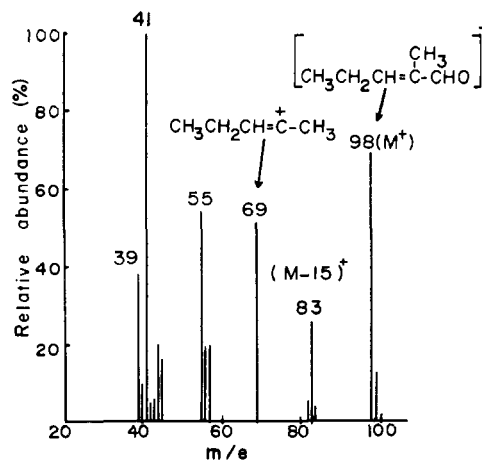


Figure 2. Mass spectrum of the volatile 10

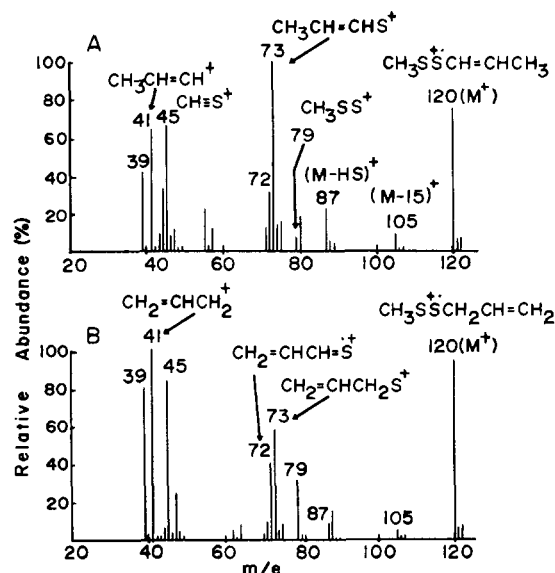


Figure 3. Mass spectra of the volatiles 14A and 15B

As a lachrymatory substance and the breakdown products have never been found so far in garlic-like alliums, the occurrence of 2-methyl-2-pentenal in caucas is of interest in terms of the chemotaxonomy.

The peak 14 (M^+ 120) and 19 (M^+ 146) are isomers of methyl allyl disulfide (peak 15) and diallyl disulfide (peak 20), respectively. The mass spectra of these isomers are shown in Figures 3 and 4.

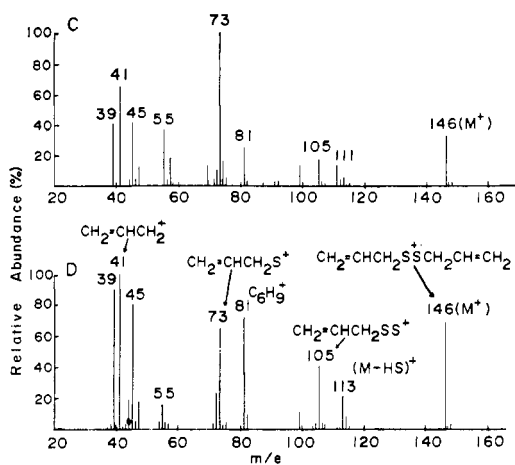


Figure 4. Mass spectra of the volatiles 19C and 20D

The fragmentations of the mass spectra were presumed on the basis of the data of the metastable ion peaks for disulfides (Bowie *et al.*, 1966) and of unsaturated disulfides (Brodnitz *et al.*, 1969). In Figure 2 the relative abundances of fragments, m/e 41, 73, 79, 87, etc., explain the remarkable differences between methyl 1-propenyl disulfide and methyl allyl disulfide; the bond of S-C (allyl group moiety) in methyl allyl disulfide is more easily cleaved than that of S-C (1-propenyl group moiety) in methyl 1-propenyl disulfide, while 1-propenylthio cation ($\text{CH}_2\text{CH}=\text{CHS}^+$ m/e 73) is more stable than allylthio cation ($\text{CH}_2=\text{CHCH}_2\text{S}^+$). From this information the peak 14 is presumed to be methyl 1-propenyl disulfide. Similarly, in Figure 3, the relative abundances of fragments, m/e 73, 81, 113, etc., show the remarkable differences between the isomers; the release of hydrothio

radical (HS) from diallyl disulfide is easier than that from 1-propenyl-containing disulfide, and a fragment, m/e 73 ($\text{CH}_2\text{CH}=\text{CHS}^+$), of the peak 19 is the base peak of the mass spectrum. From this information the peak 19 is presumed to be either allyl 1-propenyl disulfide or di-1-propenyl disulfide.

Further investigations of 1-propenyl-containing compounds and the caucas flavor precursors will be presented elsewhere.

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