Studies on the Flavor of Green Tea

Part IX. Identification and Composition of Intermediate and High Boiling Constituents in Green Tea Flavor

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By continuing flavor analysis of green tea from a previous paper,¹⁾ further twenty seven compounds were newly identified. These compounds are limonene, α -cubebene, α -copaene, caryophyllene, α -humulene, α - and γ -muurolene, β -sesquiphellandrene, δ -cadinene, calamenene, cubenol, α -cadinol, α -terpineol, *n*-heptanol, *n*-nonanol, furfuryl alcohol, *n*-nonanal, *N*-ethylformylpyrrole, pyrrolmethylketone, 6-methyl-*trans*-3,5-heptadien-2-one, 2',2''-dihydro- α -ionone, 6,10,14-trimethyl-2-pentadecanone, *cis*-3-hexenylcaproate, *cis*-3-hexenylbenzoate, α -terpinylacetate, coumarin and diphenylamine.

Relative quantities of known compounds in intermediate- and high-boiling fraction were determined.

In a previous paper,¹⁾ three alcohols, three ketones, one ester, one phenol and one nitrogenous compounds were reported as the flavor components of intermediate and high boiling fractions from green tea. But some contributory flavor components remained unknown. Therefore extensive studies on structure elucidation of the unknown components were continued by the same procedure as described in a previous paper.¹⁾

EXPERIMENTAL AND RESULTS

Same neutral oil, consisted of intermediate and high boiling compounds, as described in a previous paper¹⁾ was separated into thirteen fractions by silica-gel column chromatography. Yield of each fraction obtained from 620 mg of the oil is shown in Table I. Equipments used for separation and identification of compounds were same as described in a previous paper.¹⁾

TABLE I. FRACTIONS OBTAINED BY SILICA-GEL COLUMN CHROMATOGRAPHY

Solvent Ether content in <i>n</i> -hexane (%)	Volume (ml)	Frac- tion No.	Yield of concen- trate (mg)	Components in fraction
0	200	1-a	155	Hydrocarbons
0	200	1-b	127	
1	200	2	125	
2	200	3-a	104	
2	300	3-b	333	Oxygenated or Nitrogenous compounds
3	200	4	304	
4	200	5	316	
6	200	6	577	
8	200	7	268	
10	200	8	309	
20	200	9	293	
pure MeOH	50	10-a	228	
pure MeOH	100	10-b	2309	

Identification of the compounds was verified by the agreement of IR and mass spectra as well as gas chromatographic data with those of authentic samples or published standard references.

1) T. Yamanishi, M. Nose and Y. Nakatani, *Agr. Biol. Chem.*, **34**, 599 (1970).

Authentic compounds

cis-3-Hexenyl caproate and benzoate were synthesized by reaction of *cis*-3-hexenol with caproylchloride and benzoylchloride respectively.

3,4-Dihydro- α -ionone was obtained by hydrogenation of α -ionone over Raney Ni.

N-Ethylformylpyrrole was synthesized by the

same procedure as described by J. H. Tatum *et al.*²⁾

6-Methyl-*trans*-3,5-heptadien-2-one was synthesized from methyl- β,β -dimethylacrylate by reduction with LiAlH_4 followed by oxidation with MnO_2 and then condensation with acetone.

α -Humulene and epi-cubenol were given by

FIG. 1. Gas Chromatograms of Hydrocarbon Fractions (Fraction No. 1-a through No. 3-a).

2) J. H. Tatum, P. E. Shaw and R. E. Berry, *J. Agr. Food Chem.*, **15**, 773 (1967).

Dr. Y. Naya and by Dr. Y. Ohta of Institute of Food Chemistry, Osaka.

Other compounds came from commercial source.

Authentic sample spectrum for IR

IR spectra of authentic α -muurolene, γ -muurolene, α -copaene, α -cubebene, cubenol epi-

cubenol and α -cadinol were given by Dr. Y. Ohta above mentioned and by Dr. Y. Hirose, Professor, University of Tokyo.

Fraction of hydrocarbons (Fraction No. 1 through No. 3-a)

Fraction No. 1 through No. 3-a consisted of hydrocarbons. Gas chromatograms of these

FIG. 2. IR Spectra of Newly Identified Components in Hydrocarbon Fraction.

— Trapped sample
 ---- Authentic compound

fractions are shown in Fig. 1.

Peaks 1, 4, 7 and 10 were proved to be limonene, caryophyllene, α -muurolene and α -humulene respectively in this order on the basis of agreements of gas chromatographic data, IR and MS with those of authentic compounds.

Peaks 2, 3, 6, 8, 9 and 11 were proved to be α -cubebene, α -copaene, γ -muurolene, δ -

cadinene, calamenene and β -sesquiphellandrene respectively by interpretation of MS and comparison of IR with those of standard references reported by J. A. Wenniger *et al.*³⁾ and given by Dr. Y. Hirose and Dr. Y. Ohta. IR spectra of nine peaks of sesquiterpene hydrocarbons and several MS are shown in Fig. 2 and Fig. 3.

FIG. 3. Mass Spectra of Newly Identified Components in Hydrocarbon Fraction.

3) J. A. Wenniger, R. L. Yates and M. Colinsky, *J. A. O. A. C.*, **50**, 1313 (1967).

FIG. 4. IR and Mass Spectra of Peak 5 and Authentic 1,2,4-Trichlorobenzene.
—, Peak 5; ----, Authentic 1,2,4-trichlorobenzene

Peak 5 was proved to be 1,2,4-trichlorobenzene by agreements of IR and MS (Fig. 4) as well as gas chromatographic data with those of authentic sample. It seemed to come from pesticide.

Fraction of oxygenated compound including nitrogenous compound (Fraction No. 3-b through No. 10-b)

Gas chromatograms of Fractions No. 3-b through No. 10-b are shown in Fig. 5. Since peak 39~41 could not be well separated, those mixed peaks were trapped and rechromatographed by the use of column SE 30 (10%).

Peaks 12, 13, 14, 15, 16, 17, 19, 30, 33, 35, 38, 41, 42, 47, 50, 52, 55 and 57 were all newly identified by the agreement of IR and mass spectra as well as gas chromatographic data with those of authentic compounds. Names of corresponding compounds to each peak are written on the peaks in Fig. 5.

Peak 18 was proved to be 6,10,14-trimethyl-

2-pentadecanone by comparison of its MS with a published standard spectrum⁴⁾ as well as examination by IR. The compound has been found in coffee flavor⁴⁾ and jasmin oil.⁵⁾ It has been presumed to be produced by reaction between high molecular terpene and lipid.

Peaks 20 through 29 were reported in a previous paper.¹⁾

Both peaks 39 and 40 were presumed to be keto-alcohols with molecular weight 156 from interpretations of MS and IR.

IR and mass spectra of newly identified constituents of green tea flavor except the compounds which has been found in black tea are shown in Fig. 6 and Fig. 7.

Table II is a list of identified compounds in the intermediate and high boiling fraction from green tea aroma concentrate. Relative

4) M. Stoll, M. Winter, F. Gautschi, I. Flament and B. Willhalm, *Helv. Chim. Acta*, **50**, 628 (1967).

5) E. Demole, B. Willhalm and M. Stoll, *Helv.*, **47**, 1152 (1964).

FIG. 5. Gas Chromatograms of Oxygenated Fractions.

Column: 23% Carbowax 6000 on Chromosorb W 60/80. Temp.: 150°C Isothermal.
Carrier gas: 30 ml/min.

FIG. 6. IR Spectra of Newly Identified Components in Oxygenated Fraction Including Nitrogenous Compounds.

quantities of these compounds in Table II were obtained from calculation based on yield of each fraction (shown in Table I) and peak

area percentage on chromatogram of each fraction.

Synthetic mixture, which was prepared ac-

FIG. 7. Mass Spectra of Newly Identified Components in Oxygenated Fraction Including Nitrogenous Compounds.

cording to the proportion in Table II, quite resembled to the natural aroma concentrate. Moreover it was recognized that the synthetic mixture improved a low quality of green tea remarkably not only in aroma but also in taste.

DISCUSSION

As shown in Table II, thirteen sesquiterpenes were identified as the constituents of green tea aroma. These compounds are classified by their structures as shown in

TABLE II. IDENTIFICATION AND QUANTITATIVE VALUE OF THE AROMA CONSTITUENTS OF GREEN TEA

Peak No.	Constituent	Molecular formula	Relative quantity (%)	Method of identification		
1*	Limonene ^{a)}	C ₁₀ H ₁₆	0.46	IR	tR	
2*	α -Cubebene	C ₁₅ H ₂₄	0.44	IR	MS	tR
3*	α -Copaene	C ₁₅ H ₂₄	0.45	IR	MS	tR
4*	Caryophyllene	C ₁₅ H ₂₄	1.11	IR	MS	tR
5*	1,2,4-Trichlorobenzene	C ₆ H ₃ Cl ₃	1.24	IR	MS	tR
6*	γ -Murolene	C ₁₅ H ₂₄	0.76	IR	MS	tR
7*	α -Murolene	C ₁₅ H ₂₄	3.16	IR	MS	tR
8*	δ -Cadinene	C ₁₅ H ₂₄	9.40	IR	MS	tR
9*	Calamenene	C ₁₅ H ₂₂	1.04	IR	MS	tR
10*	α -Humulene	C ₁₅ H ₂₄	0.35	IR	MS	tR
11*	β -Sesquiphellandrene	C ₁₅ H ₂₄	0.32	IR	MS	tR
12*	1-Nonanal	C ₉ H ₁₈ O	0.49	IR	tR	
13*	<i>cis</i> -3-Hexenyl caproate	C ₁₂ H ₂₂ O ₂	0.95	IR	MS	tR
14*	α -Terpinyl acetate	C ₁₂ H ₂₀ O ₂	0.14	IR	tR	
15*	2',2''-Dihydro- α -ionone	C ₁₃ H ₂₂ O	0.40	IR	MS	tR
16*	Cubenol	C ₁₅ H ₂₆ O	1.07	IR	MS	tR
17*	<i>cis</i> -3-Hexenyl benzoate	C ₁₃ H ₁₆ O	0.57	IR	MS	tR
18*	6,10,14-Trimethyl-2-pentadecanone	C ₁₈ H ₃₆ O	0.36	IR	MS	
19*	Diphenylamine	C ₁₂ H ₁₁ N	0.58	IR	MS	tR
20	α -Ionone	C ₁₃ H ₂₀ O	0.53	IR	MS	tR
21	4-Ethylguaiaicol	C ₉ H ₁₂ O ₂	0.29	IR	tR	
22	Epi-cubenol ^{b)}	C ₁₅ H ₂₆ O	1.22	IR	MS	tR
23	Indole	C ₈ H ₇ N	3.88	IR	MS	tR
24	Acetophenone	C ₈ H ₈ O	0.40	IR	MS	tR
25	β -Ionone	C ₁₃ H ₂₀ O	2.56	IR	MS	tR
26	Nerolidol	C ₁₅ H ₂₆ O	4.00	IR	MS	tR
27	Linalool	C ₁₀ H ₁₈ O	19.88	IR	tR	
28	<i>t,t</i> -3,5-Octadien-2-one	C ₈ H ₁₂ O	0.21	IR	MS	tR
29	3,7-Dimethyl-1,5,7-octatrien-3-ol	C ₁₀ H ₁₆ O	0.60	IR	MS	tR
30*	N-Ethyl formyl pyrrole ^{a)}	C ₇ H ₉ ON	0.92	IR	MS	tR
31	Phenol	C ₆ H ₆ O	0.54	IR	tR	
32	<i>m,p</i> -Cresol	C ₇ H ₈ O	0.34	IR	tR	
33*	(2,3-Dihydrobenzofuran)	C ₈ H ₈ O	0.55	IR	MS	
34	<i>cis</i> -3-Hexenol	C ₆ H ₁₂ O	0.46	IR	tR	
35*	1-Heptanol	C ₇ H ₁₆ O	0.14	IR	tR	
36	1-Octanol	C ₈ H ₁₈ O	1.59	IR	tR	
37	(An $\alpha\beta$ -unsaturated alcohol)	C ₈ H ₁₄ O	0.10			
38*	(6-Methyl- <i>t</i> -3,5-heptadien-2-one)	C ₈ H ₁₂ O	0.13	IR	MS	tR
39	(A keto alcohol)	C ₉ H ₁₆ O ₂	0.85			
40	(A keto alcohol)	C ₉ H ₁₆ O ₂	0.72			
41*	1-Nonanol	C ₉ H ₂₀ O	0.22	IR	tR	
42*	α -Terpineol	C ₁₀ H ₁₈ O	2.99	IR	MS	tR
43	Nerol	C ₁₀ H ₁₈ O	0.57	IR	MS	tR
44	Geraniol	C ₁₀ H ₁₈ O	5.53	IR	MS	tR
45	<i>cis</i> -Jasmone	C ₁₁ H ₁₆ O	2.98	IR	MS	tR

TABLE II. Continued

Peak No.	Constituent	Molecular formula	Relative quantity (%)	Method of identification		
46	(An $\alpha\beta$ -unsaturated ketone)	$C_{15}H_{24}O$	0.72			
47*	α -Cadinol	$C_{15}H_{26}O$	0.55	IR	MS	tR
48	Linaloolmonoxide I	$C_9H_{14}O_2$	0.37	IR	tR	
49	Linaloolmonoxide II	$C_9H_{14}O_2$	1.44	IR	tR	
50*	5-Methylfurfural ^{a)}	$C_6H_6O_2$	0.15	IR	tR	
51	Benzylalcohol	C_7H_8O	3.66	IR	tR	
52*	Furfurylalcohol ^{a)}	$C_5H_6O_2$	0.53	IR	tR	
53	Linaloolmonoxide III, IV	$C_9H_{14}O_2$	2.56	IR	tR	
54	Phenylethylalcohol	$C_8H_{10}O$	0.72	IR	tR	
55*	Pyrryl methyl ketone ^{a)}	C_6H_7ON	0.86	IR	tR	
56	Dihydroactinidiolide	$C_{11}H_{16}O_2$	1.20	IR	tR	
57*	Coumarin	$C_9H_6O_2$	0.66	IR	tR	

A mark * shows that the compound was newly identified as a constituent of green tea flavor.

^{a)} shows that the compound is known constituents in black tea.

^{b)} It is not clear whether the compound, which we have obtained, is epicubenol or cadinenol.

TABLE III. CLASSIFICATION OF SESQUITERPENES IN THE AROMA CONCENTRATE FROM GREEN TEA

Skeletons of sesquiterpenes			Hydrocarbons	Alcohols
Acyclic				Nerolidol
Monocyclic	Humulanic		α -Humulene	
	Bisabolanic			β -Sesquiphellandrene
Bicyclic	Caryophyllanic		Caryophyllene	
	Cadalenic	Murolane group	α -Murolene	Cubenol
		Cadinane group	γ -Murolene	epi-Cubenol
		Others	δ -Cadinene	α -Cadinol
Tricyclic	Copanic		Calamenene	
	Cubebanic		α -Copaene	
			α -Cubebene	

Table III. Looking over Table III, some copanic or cubebanic type. It has been shown⁶⁾ interesting facts were recognized; nine of that α -copaene closely related to murolene thirteen compounds belong to either cadalenic, group, so does α -cubebene to cubenol and

6) Y. Ohta, K. Ohara and Y. Hirose, *Tetrahedron Letters*, **1968**, 4181.

epi-cubenol and the following hypothesis on the genesis of these sesquiterpenes via a germacrene type intermediate was proposed by Y. Ohta *et al.*⁶⁾

Although it is not certain whether these changes occur in tea leaves (plant) or on the manufacturing process, our findings of such cadalenic type compounds could support the above pathway. It would be interesting to examine precisely the analysis of sesquiterpenes in fresh leaves in the view points of the chemotaxonomy of Theaceae family, biogenesis of these sesquiterpenes and changes in the manufacturing process.

It is noticeable that 2',2''-dihydro- α -ionone (floral aroma, like violet together with rose) and 6-methyl-*trans*-3,5-heptadien-2-one (grassy and cinnamon like aroma) have never been reported as natural products up to now.

6,10,14-Trimethyl-2-pentadecanone (flowery warm aroma) has been found in jasmin oil⁵⁾ as mentioned above. Other components in jasmin oil such as *cis*-jasmone and indole has

been found already.^{4,7)} It is said that high grade of green tea has jasmin like flavor. Therefore it seems to be meaningful to examine if quantities of these compounds have some relationship to quality of green tea.

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7) T. Yamanishi, T. Kiribuchi, Y. Mikumo, H. Sato, A. Ohmura, A. Mine and T. Kurata, *Agr. Biol. Chem.*, **29**, 300 (1965).