

Table I. Trapped Volatiles from *Dendrobium superbum* Flowers

alcohols		ketones	
ethanol	+ <sup>a</sup>	acetone	++
linalool	++	2-pentanone	+++
esters		2-heptanone	+++
methyl acetate	++	2-nonanone	++
ethyl acetate	+++	4-phenylbutan-2-one	++
2-propyl acetate	+	2-undecanone	++
2-heptyl acetate	+	2-tridecanone	+++
2-nonyl acetate	++	2-pentadecanone	++
2-undecyl acetate	+	miscellaneous	
2-tridecyl acetate	+	acetaldehyde	+
hydrocarbons		carbon disulfide	+
toluene	+	indole	++
ethylbenzene	+		
<i>o</i> -xylene	+		
<i>p</i> -xylene	+		
limonene	+		

<sup>a</sup> Crosses indicate the relative amounts of each component in the trapped volatiles mixture.

stream by resetting the valves. The stainless steel capillary column [500 ft × 1/16-in. o.d. × 0.03-in. i.d., coated with methyl silicone oil [SF-96(50)] containing 5% Igepal CO-880] was held at 45 °C for 10 min and then was programmed at 1.2–1.3 °C/min to 182 °C, where it was held until completion of the run (40 min). The column exit was interfaced with a quadrupole mass spectrometer (Noble et al., 1980) through a silicone rubber membrane type separator (held at 180–185 °C). Mass spectral identifications were verified by retention index checks, using authentic samples of each component.

#### RESULTS AND DISCUSSION

Table I lists the compounds identified in this study. Quantitatively, the compounds in this list comprise nearly all of the volatile material trapped.

The largest compound group identified is the odd carbon numbered methyl ketones, ranging from acetone through 2-pentadecanone. The most prominent member of the group is 2-tridecanone; it is one of the two major components of the total trapped flower volatiles.

Ethyl acetate is the other major component of the trapped material and was preceded in the GC-MS run by

a trace amount of methyl acetate. The remaining acetates are esters of odd carbon number secondary alcohols, from 2-propyl to 2-tridecyl acetate. A single gap exists in the series, for no 2-pentyl acetate could be detected.

None of the corresponding free secondary alcohols were detected; the only alcohols appearing were ethanol and linalool.

The four aromatic hydrocarbons were all present at very low concentrations. It is unclear whether these were really evolved by the blossoms or whether they are atmospheric contaminants.

Two nitrogen- or sulfur-containing volatiles appeared in the trapped flower volatiles. Carbon disulfide was detected at quite low concentration, and an appreciable amount of indole was also found to be present.

Within the sensitivity limits of the experimental approach, no 4-(*p*-acetoxyphenyl)butan-2-one could be detected, nor was the *p*-hydroxy compound found. The free phenolic 4-(*p*-hydroxyphenyl)butan-2-one survives passage through the GC-MS system, so its apparent absence from the *Dendrobium superbum* volatiles sample is not thought to be due to irreversible adsorption or decomposition during mixture separation. As noted in Table I, 4-phenylbutan-2-one did appear among the sample constituents. The presence of this related compound suggests that the *p*-hydroxy and *p*-acetoxy compounds might yet be present in the flower volatiles but at very low concentrations, too low for detection in the amount of sample trapped for this study.

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## Volatile Constituents of Some Central African Black Tea Clones

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The major volatile constituents found in the aroma concentrates prepared from some tea clones of distinct China-type characteristics were similar to those reported for flavory tea produced in India and Sri Lanka. The dominant high-boiling compounds were linalool, *trans*-furanolinalool oxide, and geraniol. Levels of the undesirable low-boiling alcohols and aldehydes, however, were higher in the domestic clonal teas. An important finding was that, contrary to expectation, some of the clones exhibited flavor characteristics during the main production season in this region.

Central African black teas are generally recognized as plain teas, having none of the distinctive flavor associated with the much more valuable product grown at high elevation in certain districts in India and Sri Lanka. The

rapid growth during the main production season and the intensive nitrogen fertilizer policy are thought to preclude the development of the flavor precursors, since the biogenesis of the volatile terpenoid compounds is associated with physiological stress in the plant (Wickremasinghe, 1974). During the cool, dry production off-season, in this region, however, flavory teas are sometimes produced, particularly from leaf of China hybrid cultivars.

Previous studies have reported the volatile constituents of teas produced in India (Yamanishi et al., 1968a), Sri

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Lanka (Wickremasinghe et al., 1973), Japan (Saijo, 1977), and Russia (Kozhine and Treiger, 1973), and the relationship between volatile composition and flavor of the beverage has been recently reviewed (Howard, 1978). There has, however, been no investigation of the volatile constituents of teas produced in any of the major tea growing areas in Africa. In any attempt to improve the flavor potential of tea grown in Central Africa, by selection and breeding programs, it is essential to establish the major volatiles present. The volatile composition of a range of clones, some of which are known to produce flavor during the off-season, is examined in the present study. Teas produced both in the peak cropping period and in the off-season were investigated.

#### EXPERIMENTAL SECTION

Of the clones used in this investigation, CL 17, SL 1, SL 5, SL 9, SL 40, and SL 73 display marked China-type characteristics, while the seventh clone SFS 204 shows more Indian-type character in the *Camellia sinensis-assamica* hybrid spectrum. Leaf was manually harvested ("plucked") before 0700 h from populations of mature tea bushes cultivated according to established agronomic practices at the Nsuwazi Tea Research Station (latitude 16° S, altitude 630 m). The standard of plucking was that practised on local plantations, and the harvest predominantly consisted of flushing shoots of two or three fully expanded leaves together with the terminal bud. The experiment was conducted in mid-Jan 1980 during the 5-month main production period, during which some 80% of the annual yield is harvested. In a preliminary investigation CL 17, SL 5, SL 9, and SFS 204 were examined in late Aug 1979 during the Central African off-season.

**Manufacture.** The freshly plucked shoots were transferred to the Research Station pilot-scale production plant where black teas were manufactured by a standard procedure simulating commercial practice in this region. The leaf material was partially desiccated in an induced current of air at ambient temperature and relative humidity over a period of 20 h, so that 70% of the initial weight remained. In the tea industry this is referred to as a 70% wither and the operation is an essential prelude to processing the leaf. Batches of withered leaf were comminuted by a widely used dual-processing system. The leaf was preconditioned in a rotorvane unit and then further macerated in a mincer, which had a similar action to commercial machines of the C.T.C. or L.T.P. type (Wekhoven, 1974; Cloughley et al., 1981). The macerated leaf (the "dhool") was spread over a concrete surface to a uniform depth of 2.5 cm and allowed to ferment for 60 min in an air-conditioned room, where temperature varied in the range 20–24 °C. Formal fermentation was arrested by drying the dhool (to a moisture content of 3%) in a tray dryer designed to simulate the multistage models used in the industry. Fibre was extracted from the dried leaf, and samples of unrefined black tea were airmailed to the United Kingdom for volatile analysis at the Unilever laboratories. Tea liquors prepared by a standard method (Cloughley, 1980) were organoleptically examined by a professional tea taster according to a double-blind procedure.

**Materials and Equipment.** The following were used: a Pye G.C.V. gas liquid chromatograph fitted with an S.C.O.T. column packed with Carbowax 20M (length 50 m) and an F.I.D. detector; an all-glass Likens Nikkerson extraction apparatus; glass distillation columns (1.5 cm o.d. × 21 cm); *n*-pentane, A.R. grade (B.D.H. Chemicals, Ltd., Poole, Dorset, England).

**Preparation of Aroma Concentrate.** The Likens

Nikkerson apparatus currently used by us for routine preparation of aroma samples was set up with a 5-L sample flask and 2-L steam generator attached. A collecting flask (50 mL) was filled with 30 mL of *n*-pentane, and the bottom loop in the Likens Nikkerson apparatus was filled with distilled water. The water in the steam generator was brought to boil, and 40 g of the tea sample was placed in the sample flask, followed by 1800 mL of boiling distilled water. The collecting flask was connected to the Likens Nikkerson apparatus and was heated by means of an electric heating mantle. Steam from the generator was blown through the sample that was also heated directly by means of a flask mantle. Steam from the sample flask and pentane vapor from the collecting flask met together on the cold finger condenser and were separated and returned to their respective flasks in the bottom part of the apparatus. The extractor was run for 1 h before all the flask mantles were switched off and the apparatus was allowed to cool. The pentane in the bottom part of the apparatus and in the collecting flask was pooled and run through a separating funnel to remove any globules of water. The pentane layer (containing the tea aroma) was placed in a 50-mL round-bottomed flask, and this was connected to a small glass column packed with 5-mm diameter Fenske helices. The flask was gently heated in a bath of warm water, and the solvent was slowly distilled up the column, leaving about mL of concentrated tea aroma in the bottom. This was placed in a small glass container, and a continuous stream of pure nitrogen was blown over it to concentrate the sample in a volume of 0.5 mL exactly.

**Analysis of Volatiles.** A total of 0.2  $\mu$ L of the tea aroma in pentane was injected directly on to the GLC column. The column temperature was held at 70 °C for an initial period of 5 min. The temperature was then increased by 7 °C/min up to a final temperature of 180 °C, and this temperature was held for 10 min before cooling. The F.I.D. detector was kept at 200 °C throughout.

#### RESULTS

The seven clonal teas manufactured in January were classified into three categories on the basis of their flavor characteristics. Clones SL 1, SL 5, and SL 40 were said to exhibit true black tea flavor, while the flavor of CL 17 was described as pleasant but distinct from the other three. No true flavor was discernible in the remaining three clonal teas, and they were described as the typical plain teas expected from this growing region. The black teas SL 5 and CL 17 made in August had also been described as distinctly flavory, agreeing with established opinion about these clones during the off-season.

The volatile constituents of the aroma concentrate prepared from each tea are shown in Table I. Peak identification was based on the retention times of pure standards. Although there were some differences in the absolute amounts of volatiles (estimated as peak height), the first category of flavory clones shows a similar pattern in the distribution of the major peaks. While linalool was clearly the dominant volatile, there were also relatively large amounts of geraniol present.

The major low-boiling compound in these clones, and indeed in all the clones studied, was *trans*-2-hexenal. The aroma concentrates of CL 17 was also rich in linalool but different from the first group of clones in possessing high levels of the linalool oxide and comparatively minor amounts of the fragrant terpenoid geraniol.

The composition and distribution of the volatiles in the nonflavory clones differed markedly from the profile of the

Table I. Volatile Profile of Seven Clonal Teas Manufactured in Mid-January

peak no.	peak assignment	peak height, mm							estimated area, % <sup>a</sup>						
		SL 1	CL 5	SL 40	CL 17	SL 9	SL 73	SFS 204	SL 1	SL 5	SL 40	CL 17	SL 9	SL 73	SFS 204
1	<i>n</i> -hexanal	17	9	9	27	35	10	20	5	4	6	8	10	9	8
2	<i>trans</i> -2-hexenal	67	43	29	45	144	24	48	20	19	19	13	41	20	20
3	<i>cis</i> -3-hexenol	17	13	6	8	19	9	14	5	6	4	2	5	8	6
4	<i>trans</i> -2-hexenol	5	2	7	9	9	5	9	2	1	5	2	2	4	4
5	<i>trans</i> -furanolinalool oxide	15	19	27	72	10	16	21	5	9	18	21	3	14	9
6	linalool	113	73	38	118	69	36	100	34	33	25	35	20	30	41
7	phenylacetaldehyde	12	16	7	21	15	6	17	3	7	4	6	4	5	7
8	neral														
9	methyl salicylate	17	10	7	16	7	3	14	5	4	4	5	2	2	6
10	nerol														
11	geraniol	68	34	19	22	42	9		20	15	13	7	12	8	
12	$\beta$ -ionone														
13	nerolidol	4	4	2	4	tr <sup>b</sup>	tr	tr	1	2	1	1			

<sup>a</sup> The area of each peak (measured with a planimeter) expressed as a percentage of the total area of all the peaks. <sup>b</sup> tr, trace.

Table II. Volatile Profile of Clonal Teas Manufactured in Mid-August

peak no.	peak assignment	peak height, mm				estimated area, % <sup>a</sup>			
		SL 5	CL 17	SL 9	SFS 204	SL 5	CL 17	SL 9	SFS 204
1	<i>n</i> -hexanal	10	44	48	43	4	4	10	10
2	<i>trans</i> -2-hexenal	22	167	176	128	9	16	37	29
3	<i>cis</i> -3-hexenol	12	20	24	24	5	2	5	5
4	<i>trans</i> -2-hexenol	2	20	7	11	1	2	1	2
5	<i>trans</i> -furanolinalool oxide	45	211	15	34	18	20	3	8
6	linalool	54	252	43	126	22	24	9	28
7	phenylacetaldehyde	15	40	11	29	6	4	2	6
8	neral		73	2	8		7		2
9	methyl salicylate	11	86	15	19	4	8	3	4
10	nerol	2	16	3	2	1	2	1	
11	geraniol	69	86	123	13	28	9	26	3
12	$\beta$ -ionone		6	2	3		1		
13	nerolidol	2	20	9	6	1	2	2	2

<sup>a</sup> The area of each peak (measured with a planimeter) expressed as a percentage of the total area of all the peaks.

flavory clones. There were also distinct differences among the three clones in this group. SL 9 contained a smaller percentage of linalool and its oxide and reduced levels of every compound with retention times greater than that peak 4, except for geraniol, when compared to the flavory clones. By far the most dominant peak was that of *trans*-2-hexenal, which accounted for over 40% of the total volatiles present. In contrast, SL 73 contained relatively high levels of linalool and linalool oxide but only comparatively minor amounts of geraniol. Although *trans*-2-hexenal was the major low-boiling compound present, it comprised only 20% of the total volatiles, an amount comparable with that found in the flavory clones. However, the levels of the other low-boiling compounds (peaks 1, 3, and 4) were much greater than in the latter group of clones. Although SFS 204 contained the highest percentage of linalool found in the teas manufactured in January, it contained no geraniol. As was the case with SL 73, the levels of *trans*-2-hexenal were similar to those found in the flavory clones.

It can be seen in Table II that the levels of most of the volatiles were generally higher in the teas manufactured in August. Also there were more volatiles present with retention times greater than the low-boiling compounds.

In the flavory clone SL 5, the major difference between the teas manufactured on the two occasions was that the relative contribution of the linalool and geraniol peaks to the total peak area was reversed. In the off-season tea, geraniol was the dominant volatile. Another difference was the reduction in the amount and proportion of the low-boiling volatiles, especially of *trans*-2-hexenal, in the August manufacture.

In CL 17, while the amounts of linalool, linalool oxide, and phenylacetaldehyde have doubled, their proportion in the aroma concentrate was actually less than in the January teas. However, the off-season tea contained both higher levels and greater proportions of all the other higher boiling volatiles. Moreover, neral, nerol, and  $\beta$ -ionone were not present in the main-season tea but together accounted for 10% of the total volatiles in the August tea.

The seasonal differences in SL 9 showed some similarity to SL 5 in that the relative proportion of linalool was lower, and that of geraniol, higher, than in the tea produced in January. However, there was little difference in the levels of the low-boiling aldehyde and alcohols on the two occasions, *trans*-2-hexenal dominating the volatiles profile.

The volatile composition of the off-season tea made from SFS 204 differed only slightly from that of the main season. The proportion of linalool was reduced, and although the number of higher boiling volatiles was increased, they still made a minor contribution to the total volatile material.

When the ratio of low-boiling volatiles to high-boiling volatiles is considered (Table III), it can be seen that there is a relationship between this ratio and flavor—a low value indicating a flavory tea. There was a trend in the results to suggest that this value was influenced by season. In the off-season the ratio was reduced. This was true for three out of the four clonal comparisons, SFS 204 being the exception.

## DISCUSSION

The volatile constitutions of the four flavory clones studied in this investigation were similar to those reported

Table III. Comparison of the Amount and Distribution of the Volatiles in Teas Produced in January and August

clone	January		August	
	sum of peak heights, mm	ratio of peak areas <sup>a</sup>	sum of peak heights, mm	ratio of peak areas <sup>a</sup>
SL 1	335	0.47		
SL 5	223	0.43	246	0.23
SL 40	152	0.49		
CL 17	335	0.36	1051	0.35
SL 9	352	1.38	478	1.13
SL 73	118	0.67		
SFS 204	244	0.64	446	0.89

<sup>a</sup> The total estimated peak area of the low-boiling volatiles (peaks 1-4) divided by the total estimated peak area of the higher boiling volatiles (peaks 5-13).

for high-grown, flavory teas from Sri Lanka (Wickremasinghe et al., 1973) and India (Yamanishi et al., 1968a) in that linalool, *trans*-furanolinalool oxide, and geraniol were the dominant high-boiling volatiles. These terpenoid alcohols have fragrant floral odors (Yamanishi et al., 1968b) and have been generally associated with flavor in high-quality teas (Saijo, 1973; Howard, 1978). An important difference observed, especially in the SL flavory clones, was the high proportion of geraniol, which is said to have a roseline aroma (Yamanishi et al., 1968b). The major difference found, however, between the flavory tea used in the present investigation and the quality of the Sri Lanka and Indian teas was in the relative proportions of the low-boiling volatiles (peaks 1-4). The percentage of these compounds was very much higher in the Central African teas clones, even in those teas producing flavor in the off-season period. The odor of *trans*-2-hexenal is pungent and has been described as "grassy" and "beany" and is known to be deleterious to tea flavor (Yamanishi et al., 1968a; Howard, 1978).

An interesting result of the present work was the demonstration that the clone with the most pronounced *assamica* character differed from the other clones of distinct *sinensis* characteristics in the relative production of the fragrant monoterpene alcohols, linalool and geraniol. In SFS 204 linalool accounted for a much higher percentage of the total volatile material than in CL 17 or in any of the SL clones. While no geraniol was found in the tea prepared for SFS 204 in January and only a minor amount (3%) in the July tea, the percentage in the China-type clones ranged from 7 to 20% in the January teas and from 9 to 28% in the off-season teas. This agrees with the recent Japanese findings (Takeo, 1981) that *assamica* clones produce mainly linalool and *sinensis* clones mainly geraniol.

It is generally accepted (Howard, 1978) that the ratio of the sums of peak areas emerging before linalool oxide to those emerging after is important in determining tea flavor. Teas rich in the higher boiling volatiles and giving a low value for this volatiles ratio would be expected to be flavory teas. This relationship and its converse were both found to be true for the range of clones studied. The seasonal effect on the value of this volatile ratio is also of interest. Lower values were obtained during the off-season, the traditional period for flavor production in this region. Growth of tea shoots is slow during this period, and the clear, cool, dry conditions subject the plant to the same kind of climatic stress, which is responsible for the production of flavory teas in Sri Lanka and India by the mechanism proposed by Wickremasinghe (1974). The

ratio of extrachloroplastic to intrachloroplastic photosynthesis controls the relative amounts of the desirable odoriferous terpenoids (formed via the leucine pathway) and the undesirable low-boiling C<sub>6</sub> compounds, such as *trans*-2-hexenal (derived from acetate via linolenic acid). Climatic stress induces changes in the permeability of the chloroplast and promotes the extrachloroplastic leucine route.

An important finding of the present investigation was that flavor was produced in some of the China-type clones during the good growing conditions of the peak cropping period. Although the number and concentration of the higher boiling volatiles were lower and the volatiles ratio was higher than for the off-season teas, the teas were still perceived as flavory by the taster. This suggests that to some extent development of flavor and rapid growth are not necessarily mutually exclusive. It is possible that further screening and selection of clonal material may discover a genotype that would combine better flavor potential with acceptable crop production. Another approach to the problem is suggested from recent work on composite tea plants (Kayange et al., 1981). It was found that the rootstock largely controlled the green leaf yield, while the scion was the dominant influence on the character of the black tea. Selection of suitable rootstock and scion components may then produce a flavory, high-yielding composite plant. Finally, it should be pointed out that the manufacturing systems used in this region have been devised to optimize the production of theaflavin (Cloughley, 1978; Cloughley and Ellis, 1980; Cloughley et al., 1981), which is responsible for the desirable characteristics and value of plain teas (Hilton and Ellis, 1972; Cloughley, 1980). It has been shown in preliminary work (Pendlington and Humphrey, 1981) with teas from Assam that the more intensive processing machines, such as the C.T.C. and L.T.P. units used widely in this region, produce teas poorer in flavor than the same teas processed by the orthodox roller. Clearly, the influence of manufacturing procedures on the development of flavor deserves critical study.

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