

ARTICLES

Essential Oil Constituents of Ashanti Pepper (*Piper guineense*) Fruits (Berries)

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The composition of the volatile oil from the fruits (berries) of Ashanti pepper (*Piper guineense*) was investigated in detail by means of gas chromatography (GC) and gas chromatography-mass spectrometry techniques. The phenylpropanoid-rich essential oil was comprised of myristicin, sarisan, safrole, and elemicin as the dominant components. Fifty-one mono- and sesquiterpenoids constituted a minor proportion of the oil. Considering the plants dietary and medicinal use in West Africa and given the relatively high concentration of benzodioxole derivatives, the potential toxicity of the volatile oil is briefly evaluated.

Ashanti or West African black pepper (*Piper guineense* Schum and Thom) is a herbaceous climber commonly found in the African tropical forest zones. The fruits or berries are usually on sale in the local markets as a condiment and for food flavoring. Some degree of nutritive value was earlier associated with the plant in Nigeria (Ifon and Bassir, 1979, 1980). According to Guenther (1951), *P. guineense* belongs to the group of peppers called false cubebs, and the berries are often used as substitutes for the edible black pepper (*Piper nigrum*). Furthermore, the fruits of Ashanti pepper are widely employed in the West African indigenous medical system as a remedy for rheumatism and bronchitis (Irvine, 1961).

P. guineense berries, like other types of pepper, yield an aromatic essential oil on steam distillation. Nothing is known about the chemical composition of this volatile oil. In view of the widespread dietary and medicinal use of the plant, it was desirable to acquire comprehensive knowledge on the volatile oil composition. The purpose of this paper is to report the results of the first detailed GC and GC-MS analyses of the essential oil of Ashanti pepper fruits.

EXPERIMENTAL SECTION

Ripe fruits (berries, 200 g) of *P. guineense* were collected from mature plants growing in Wanikin village, Oyo State, Nigeria. Taxonomic authentication of the plant was conducted by A. Adesakin, Department of Pharmacognosy, Obafemi Awolowo University, Ife. Fresh fruits were homogenized with a small amount of water, and the slurry was steam-distilled in an all-glass apparatus designed to the specifications of *British Pharmacopoeia* (1980) for 4 h. The light yellow volatile oil possessing a characteristic spicy aroma was recovered in 0.35% (w/w) yield.

The volatile oil (70 μ L) was fractionated by means of liquid chromatography on a SEP-PAK silica gel column (2.5 cm \times 1.0 cm (o.d.)). Elution with *n*-pentane (3 mL) followed by ethyl acetate (3 mL) gave the hydrocarbon and oxygenated fractions, respectively. The solvents were evaporated from the extracts under a gentle stream of nitrogen at room temperature.

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Gas chromatographic analyses of the essential oil and the two fractions were performed on a Dani 3865 HR GC equipped with a FID and PTV (programmed-temperature vaporizer) injector. Three different fused silica capillary columns coated with BP 1 (50 m \times 0.33 mm (i.d.)), OV 351 (25 m \times 0.33 mm (i.d.)), and SE 54 (20 m \times 0.33 mm (i.d.)) with 0.5- μ m phase thickness were used. Subsequent operating GC conditions in the runs were identical with the individual columns. PTV injector temperature was increased from 55 to 250 $^{\circ}$ C in 9 s, and oven temperature was programmed from 65 $^{\circ}$ C with a 2-min initial hold and then 5 $^{\circ}$ C/min increase to a final temperature of 220 $^{\circ}$ C. Carrier gas was hydrogen at an inlet pressure of 0.22 bar, and the split ratio was maintained at 40:1. Injection volume was typically 0.5 μ L.

The GC system was linked to a Shimadzu CR 3A Chromatopac integrator for computation of peak areas. Kovats retention indices (KI) (Kovats, 1958) were obtained from the data on the relative retention behavior of the *n*-alkane homologous series.

Computerized gas chromatography-mass spectrometry (GC-MS) analytical runs were conducted on a Hewlett-Packard HP 5890 GC interfaced with a quadrupole mass spectrometer (HP 5970). Working GC conditions: columns, BP 1 (50 m \times 0.25 mm (i.d.)), OV 351 (25 m \times 0.25 mm (i.d.)), and SE 54 (15 m \times 0.20 mm (i.d.)); oven temperature program, 70-220 $^{\circ}$ C at 5 $^{\circ}$ C/min; carrier gas, helium at 0.5 mL/min. Mass spectra were taken with the ionization voltage of 70 eV and electron multiplier energy of 1600 V. Scan rate was 1100 amu/s, and the vacuum was maintained at 1.6×10^{-5} Torr with the ion source temperature at 250 $^{\circ}$ C. MS data were recorded and processed by an on-line desktop computer (HP 9825B) equipped with a Disc Memory (HP 9134).

Identification of Constituents. Individual components in the volatile oil were identified by comparison of the MS data with those of authentic standards held in the computer library and the literature (von Sydow et al., 1970; Stenhagen et al., 1974; Ohloff and Pawlak, 1970). The data in the computer library were obtained from the mass spectra of reference samples, which were gifts, were purchased from commercial sources, or were identified constituents of other essential oils of known composition (e.g., needle oil of *Pinus sylvestris*). Only similarity indices (SI) of 0.9 or higher were acceptable as proof of identity (Laakso et al., 1986). Additional literature Kovats retention indices values (Jennings and Shibamoto, 1980) and those of reference samples were used for confirmation of identification.

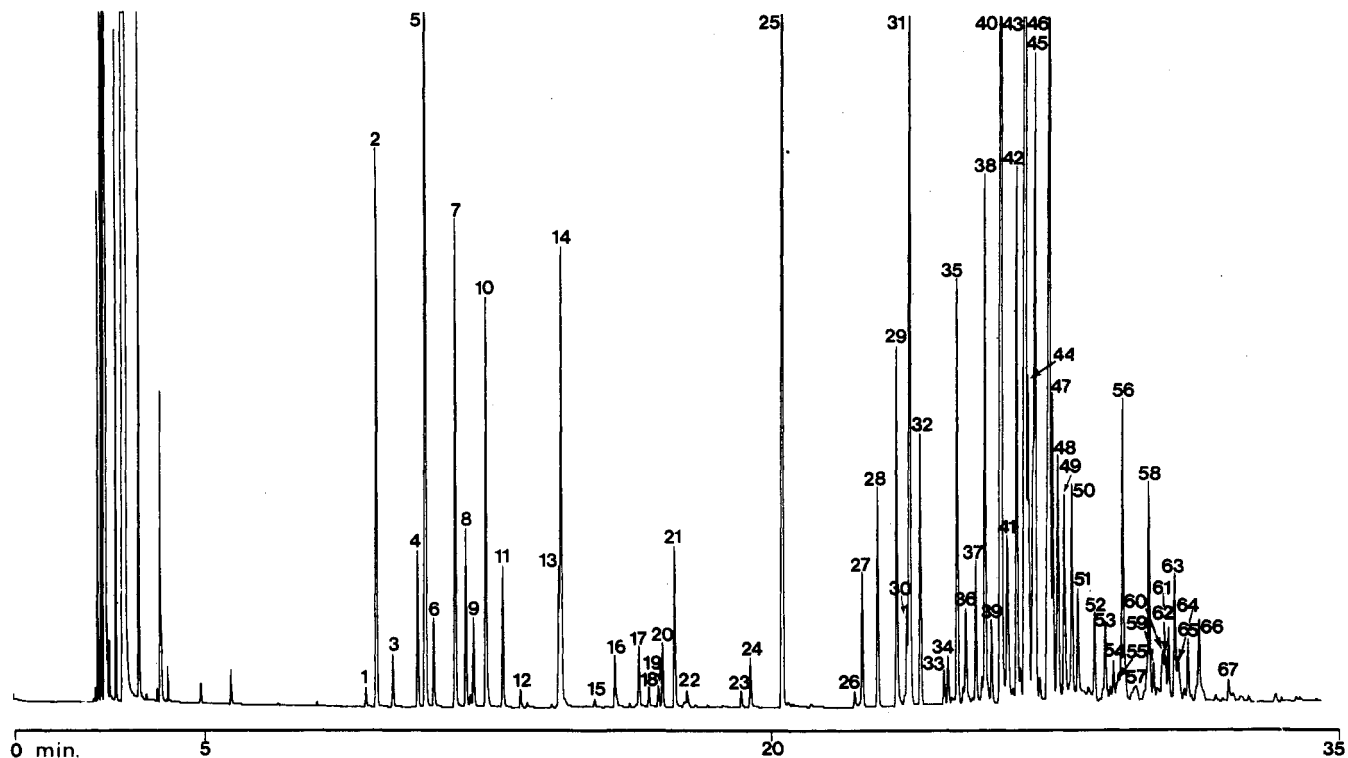


Figure 1. Gas chromatogram of the volatile oil of Ashanti pepper (*P. guineense* fruits: column, fused silica coated with BP 1 (50 m \times 0.33 mm (i.d.), 0.5- μ m phase thickness); column temperature, 65 $^{\circ}$ C for 2 min and then 5 $^{\circ}$ C/min to 220 $^{\circ}$ C; carrier gas, hydrogen at 0.22 bar. For the identity of the numbered peaks, refer to Table I.

Table I. Composition of the Volatile Oil of Ashanti Pepper (*P. guineense*)

GC peak no.	KI ^a	mass spectral data ^b	% compn	compound	GC peak no.	KI ^a	mass spectral data ^b	% compn	compound
1	930	93, 91, 77, 92, 41	0.07	α -thujene	35	1430	41, 93, 79, 69, 91	1.82	β -caryophyllene
2	938	93, 91, 92, 77, 79	1.98	α -pinene	36	1441	161, 105, 91, 41, 79	0.52	C ₁₅ H ₂₄ ^d
3	953	93, 121, 41, 67, 79	0.19	camphene	37	1455	41, 69, 93, 133, 79	0.63	(<i>E</i>)- β -farnesene
4	973	93, 91, 77, 41, 136	0.57	sabinene	38	1465	161, 105, 119, 81, 91	2.28	C ₁₅ H ₂₄ ^d
5	978	93, 41, 67, 79, 91	4.90	β -pinene	39	1471	93, 41, 80, 121, 91	0.39	α -humulene
6	985	41, 69, 93, 53, 91	0.33	myrcene	40	1481	192, 147, 53, 119, 91	15.70	risaran
7	1000	93, 91, 77, 92, 136	1.85	α -phellandrene	41	1486	161, 105, 43, 119, 107	1.01	C ₁₅ H ₂₄ ^d
8	1011	93, 91, 77, 79, 41	0.67	car-3-ene	42	1487	161, 105, 41, 91, 119	2.31	germacrene D
9	1018	119, 91, 93, 121, 43	0.34	<i>p</i> -cymene	43	1500	192, 91, 119, 65, 165	16.55	myristicin
10	1030	{ 67, 66, 93, 41, 53 93, 91, 77, 136, 79 }	1.61	limonene	44	1506	41, 69, 93, 79, 67	1.39	β -bisabolene
11	1042	93, 79, 41, 91, 77	0.56	β -phellandrene	45	1516	208, 193, 133, 77, 105	3.91	elemicin
12	1062	93, 91, 77, 136, 43	0.07	(<i>Z</i>)- β -ocimene	46	1530	159, 129, 128, 131, 160	5.69	calamenene
13	1085	93, 121, 136, 91, 79	0.55	γ -terpinene	47	1533	161, 119, 105, 134, 91	1.31	δ -cadinene
14	1087	43, 41, 71, 55, 93	1.87	terpinolene	48	1539	119, 105, 161, 41, 121	1.33	cadina-1,4-diene
15	1114	43, 71, 41, 55, 93	tr	linalool	49	1545	59, 93, 43, 161, 107	1.11	elemol
16	1132	95, 41, 81, 55, 69	0.25	(<i>Z</i>)-sabinene hydrate	50	1552	41, 43, 69, 93, 55	1.11	nerolidol
17	1152	95, 41, 43, 110, 93	0.25	camphor	51	1557	119, 43, 159, 81, 131	0.68	ui
18	1160	95, 41, 110, 43, 67	0.10	isoborneol	52	1573	121, 43, 93, 78, 66	0.46	ui
19	1168	43, 135, 91, 119, 65	0.10	L-borneol	53	1583	43, 55, 69, 78, 81	0.53	ui
20	1171	71, 43, 93, 41, 55	0.27	<i>p</i> -cymen-8-ol	54	1587	41, 43, 79, 93, 69	0.22	caryophyllene epoxide
21	1181	59, 43, 93, 121, 81	0.66	terpinen-4-ol	55	1595	208, 193, 105, 133, 41	0.21	isoelemicin
22	1190	91, 92, 81, 41, 43	0.04	α -terpineol	56	1600	161, 59, 107, 105, 91	1.70	guaial
23	1238	82, 110, 41, 95, 109	0.08	ui ^c	57	1613	222, 207, 177, 149, 121	0.09	dill apiole
24	1246	152, 123, 77, 79, 91	0.20	piperitone	58	1628	43, 91, 119, 205, 93	1.30	spathulenol
25	1273	162, 104, 131, 103, 51	4.81	ui	59	1638	43, 95, 161, 121, 79	0.20	T-muurolol
26	1338	164, 149, 131, 77, 103	0.07	safrrole	60	1645	161, 41, 43, 119, 105	0.14	cubenol
27	1345	121, 93, 136, 41, 161	0.58	eugenol	61	1647	43, 95, 121, 93, 105	0.58	α -cadinol
28	1359	105, 119, 161, 91, 41	0.94	δ -elemene	62	1651	59, 107, 135, 161, 93	0.37	bulnesol
29	1376	178, 91, 103, 107, 147	1.53	α -cubebene	63	1658	159, 91, 118, 117, 131	0.67	cadina-1,4-diene-3-ol
30	1385	167, 182, 139, 124, 109	0.44	methyleugenol	64	1662	43, 41, 119, 69, 109	0.33	α -bisabolol
31	1388	105, 119, 161, 41, 93	3.87	ui	65	1672	105, 43, 41, 91, 106	0.35	ui
32	1397	81, 93, 67, 68, 107	1.15	β -elemene	66	1684	68, 41, 122, 121, 59	0.68	ui
33	1422	41, 93, 119, 69, 91	0.17	(<i>E</i>)- α -bergamotene	67	1716	41, 91, 81, 132, 43	0.11	ui
34	1426	105, 161, 119, 91, 189	0.23	α -gurjunene					

^a KI, Kovats retention indices on BP 1. ^b Major fragmentation ions, base peak and three other ions in decreasing order of relative abundance, M⁺ = molecular ion. ^c ui = unidentified constituent. ^d C₁₅H₂₄ = unidentified sesquiterpenoid hydrocarbon.

RESULTS AND DISCUSSION

Figure 1 shows a typical chromatogram for the volatile oil of Ashanti pepper (*P. guineense*) fruits on a BP 1 coated capillary column. Table I gives the details of the components of the oil along with GC and MS data that were helpful in their identification. The percentage compositions of the compounds from summation of the peak areas are also included in this table. Exploratory GC-MS analyses revealed considerable overlapping among the numerous constituents. In our quest for a reasonably complete identification of the volatiles, the oil was fractionated into hydrocarbon and oxygenated parts. Capillary GC and GC-MS analyses were performed on three stationary phases of different polarities, and many coeluting compounds were resolved with certainty by these procedures. The bonded-phase column, BP 1, was used in most of the analytical runs because of its relatively high efficiency.

Altogether, 56 compounds amounting to about 90% of the essential oil were identified. The oil was very rich in phenylpropanoid derivatives, and myristicin was the most abundant constituent. Other analogous components in the oil were sarisan, safrole, elemicin, methyleugenol, eugenol, dill apiole, and isoelemicin. The total quantitative contribution of these compounds to the oil amounted to 43%. The minor constituents were predominantly mono- and sesquiterpenoids. Of the 14 monoterpenoid hydrocarbons, the prominent components were α - and β -pinene, α -phellandrene, and limonene. An identical number of sesquiterpenoid hydrocarbons made up a moderate fraction of the terpenoid composition. Calamene, α -copaene, and germacrene D were the main constituents. The oxygenated mono- and sesquiterpenoid contents were relatively small, and among these compounds, linalool, guaiol, and spathulenol, were found in significant amounts. Three sesquiterpenoid alcohols, bulnesol, guaiol, and cadina-1,4-dien-3-ol, have not been reported to be constituents of *Piperaceae* essential oils.

In general, Ashanti pepper oil shows remarkable quantitative similarities to the volatile oil of nutmeg (*Myristica fragrans*) (Forrest and Heacock, 1972; Schenk and Lamparsky, 1981). However, the myristicin content was found to be much higher in the Ashanti pepper oil, and sarisan has not been reported as a component of nutmeg oil yet. Sarisan was identified in the volatile oil of *Beilschmiedia miersii*, an avocado-related plant from Chile (Kumamoto and Scora, 1970). The mono- and sesquiterpenoid compositions of the *P. guineense* oil resemble to a certain extent that of black pepper (Lawrence, 1985; Debrauwere and Verzele, 1976; Richard et al., 1971), but the phenylpropanoid contents differ considerably. The spicy aroma of the Ashanti pepper oil may originate from the combination of mono- and sesquiterpenoids with myristicin.

The toxic and psychotropic properties of myristicin (the main constituent of Ashanti pepper oil) have been reviewed (Shuglin, 1966; Forrest and Heacock, 1972). Carcinogenic activities of safrole and the adverse pharmacological effects of elemicin are also well-known (Singleton and Kratzer, 1969). Dill apiole and myristicin were found to be toxic to insects in addition to being synergists for some insecticides (Lichtenstein et al., 1974). Presently, there are no toxicological or pharmacological data on sarisan, but it seems quite possible that the compound may show myristicin-like activities on account of structural isomerism. Although the principal phenylpropanoids in the volatile constitute about 0.15 $\mu\text{g/g}$ of the fruits, it is conceivable to presume attendant potential toxicity on ingestion.

Registry No. α -Thujene, 2867-05-2; α -pinene, 80-56-8; camphene, 79-92-5; sabinene, 3387-41-5; β -pinene, 127-91-3; myrcene, 123-35-3; α -phellandrene, 99-83-2; car-3-ene, 13466-78-9;

p-cymene, 99-87-6; limonene, 138-86-3; β -phellandrene, 555-10-2; (*Z*)- β -ocimene, 3338-55-4; γ -terpinene, 99-85-4; terpinolene, 586-62-9; linalool, 78-70-6; sabinene hydrate, 546-79-2; camphor, 76-22-2; isoborneol, 124-76-5; *l*-borneol, 464-45-9; *p*-cymen-8-ol, 1197-01-9; terpinen-4-ol, 562-74-3; α -terpineol, 98-55-5; piperitone, 89-81-6; safrole, 94-59-7; eugenol, 97-53-0; δ -elemene, 20307-84-0; α -cubebene, 17699-14-8; methyleugenol, 93-15-2; α -copaene, 3856-25-5; β -elemene, 515-13-9; α -bergamotene, 17699-05-7; α -gurjunene, 489-40-7; β -caryophyllene, 87-44-5; (*E*)- β -farnesene, 18794-84-8; α -humulene, 6753-98-6; sarisan, 18607-93-7; germacrene D, 23986-74-5; myristicin, 607-91-0; β -bisabolene, 495-61-4; elemicin, 487-11-6; calamenene, 483-77-2; δ -cadinene, 483-76-1; cadina-1,4-diene, 29837-12-5; elemol, 639-99-6; nerolidol, 142-50-7; caryophyllene epoxide, 1139-30-6; isoelemicin, 5273-85-8; quaiol, 489-86-1; dill apiole, 484-31-1; spathulenol, 6750-60-3; T-murolol, 19912-62-0; cubenol, 21284-22-0; α -cadinol, 481-34-5; bulnesol, 22451-73-6; cadina-1,4-dien-3-ol, 114791-16-1; bisabolol, 515-69-5.

LITERATURE CITED

- British Pharmacopoeia*; H.M.S. Stationery Office: London, 1980; Vol. II, P.A. 109.
- Debrauwere, J.; Verzele, M. J. "Constituents of Peppers IV. The Hydrocarbons of Pepper Essential Oil". *J. Chromatogr. Sci.* 1976, 14, 296.
- Forrest, J. E.; Heacock, R. A. "Nutmeg and Mace, The Psychotropic Spices from *Myristica fragrans*". *Lloydia* 1972, 35, 440.
- Guenther, E. *The Essential Oils*; van Nostrand: New York, 1950; Vol. IV, p 156.
- Ifon, E. T.; Bassir, O. "The Efficiency of Utilising the Iron in Leafy Green Vegetables for Hemoglobin Synthesis by Anemic Rats". *Food Chem.* 1979, 4, 163.
- Ifon, E. T.; Bassir, O. "The Nutritive Value of Some Nigerian Leafy Green Vegetables. Part 2. The Distribution of Protein, Carbohydrates (including Ethanol-Soluble Simple Sugars), Crude Fat, Fibre and Ash". *Food Chem.* 1980, 5, 231.
- Irvine, F. R. *Woody Plants of Ghana*; Oxford University: London, 1961; p 40.
- Jennings, W.; Shibamoto, K. *Quantitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography*; Academic: New York, 1980.
- Kovats, E. "Gas-chromatographische Charakterisierung Organischer Verbindungen. Teil 1. Retentionsindices Aliphatischer, Halogenide, Alkohole, Aldehyde und Ketone". *Helv. Chim. Acta* 1958, 41, 1915.
- Kumamoto, J.; Scora, R. N. "The Structure of Sarisan. An Isomer of Myristicin, Isolated from the Leaf Oil of *Beilschmiedia miersii*". *J. Agric. Food Chem.* 1970, 18, 544.
- Laakso, I.; Hiltunen, R.; Seppänen, T. "The Reliability of Similarity Indices for Comparing Spectra Data in GC-MS Analysis". In *Progress in Essential Oil Research*; Brunke, E.-J., Ed.; Walter de Gruyter: Berlin, 1986; p 619.
- Lawrence, B. M. "Progress in Essential Oil". *Perfum. Flavor.* 1985, 10, 52.
- Lichtenstein, E. P.; Liang, T. T.; Schulz, K. R.; Schnoes, H. K.; Carter, G. T. "Insecticidal and Synergistic Components Isolated from Dill Plants". *J. Agric. Food Chem.* 1974, 22, 658.
- Ohloff, G.; Pawlak, M. "Saurekatalysierte Isomerisierung von (-)- α -Copaen-epoxide". *Helv. Chim. Acta* 1970, 53, 245.
- Richard, H. M.; Russell, G. F.; Jennings, W. G. "Volatile Components of Black Pepper Varieties". *J. Chromatogr. Sc.* 1971, 9, 560.
- Schenk, H. P.; Lamparsky, D. "Analysis of Nutmeg Oil using Chromatographic Methods". *J. Chromatogr.* 1981, 204, 391.
- Shuglin, A. T. "Possible Implication of Myristicin as a Psychotropic Substance". *Nature (London)* 1966, 210, 380.
- Singleton, V. L.; Kratzer, F. H. "Toxicity and Related Physiological Activity of Phenolic Substances of Plants Origin". *J. Agric. Food Chem.* 1969, 17, 497.
- Stenhagen, E.; Abrahamsson, S.; McLafferty, F. W. *Atlas of Mass Spectral Data*; Wiley: New York, 1974.
- Von Sydow, E.; Anjou, K.; Karlsson, G. "Monocyclic, Dicyclic Tricyclic Terpenes". *Arch. Mass. Spectral Data* 1970, 1, 387.

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