CHEMICAL CONSTITUENTS OF FRAGRANT CAPSICUMS OF NIGERIA – PART III.

THE OXYGENATED FRACTION OF THE ESSENTIAL OIL OF THE NSUKKA YELLOW PEPPER.

BY

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ABSTRACT

The highly fragrant acid – free oxygenated fraction of the essential oil of the Nsukka Yellow Pepper contains at least 116 components (detectable on Capillary GC) made up of free alcohols (3.8%) and aliphatic esters (> 91%). A total of 37 esters and four free alcohols were found of which only 6 esters had earlier been reported in C. fruitescens, the other compounds being new to the genus Capsicum. The most abundant component of this fraction was butanoic acid, 3-methyl-, 4-methylpentyl ester which accounted for 22.2% of the oil.

INTRODUCTION

The Nsukka Yellow Pepper has been described as a pepper with "a unique aroma which is not known to exist in any other pepper cultivar". (2). Apart from the extra ordinary flavour which has been tagged the "Nsukka Yellow Aroma" (2), this pepper which is yellow when ripe is also the most pungent pepper in Nigeria. The Nsukka Yellow aroma resides in the essential oil of the pepper. (7). The pepper grows freely only within about 10km radius of the university town of Nsukka in Eastern Nigeria. These qualities and the scarcity value combine to make the Nsukka Yellow Pepper the most expensive pepper on the Nigerian Market – costing more than 10k per fruit even in season. Our interest in the chemical study of the essential oil from this drug was stimulated by the possibility of using the fragrant principles as flavouring agents for food and drugs. Part II of the study (1) deal with the chemical composition of the hydrocarbon fraction of the essential oil. We now report our findings in a chemical investigation of the oxygenated fraction which is responsible for most of the distinctive flavour of the essential oil.

EXPERIMENTAL

Materials

Fresh ripe Nsukka Yellow Peppers (*C. annuum.* L. var. OS/UN/60 Nsukka Yellow) were purchased from local markets in Nsukka between June and September, 1986.

Authentic chemicals used for confirmation of identities of the components of the oxygenated fraction were synthesised in our laboratories in Benin from materials purchased from Aldrich Chemical Company Limited or obtained as such from the same supplier. The purity of each authentic compound was confirmed by capillary gas chromatography.

Isolation of the Oxygenated fraction:

Two kilograms of fresh ripe Nsukka yellow pepper was ground coarse in a Moulinex blender 2 and extracted by maceration during 3 days at room temperature with 1.4 L of a mixture of fractionated n-hexane and diethylether (1:1). Steam distillation of the resulting concentrated extract during 4 hours gave a fragrant yellow oil (1.34g) with the typical aroma of the Nsukka Yellow Pepper.

Isolation of the oxygenated fraction was achieved by middle pressure liquid chromatography (MPLC) of the essential oil on 88g of Machery and Nagel silica gel, mesh 60, using Buchi 682 chromatography pump and eluting with 0.5% w/v NaOH to give 0.86g of a yellow oil with fragrance reminiscent of the Nsukka Yellow aroma.

The oil was subjected to GC – MS analysis and also to analytical capillary GC measurement with and without internal hydrocarbon standards to enable the calculation of retention indices (RI).

$$RI = 100.Z + (100.\frac{Rt_x - Rt_z}{Rt_{z+i} - Rt_z})$$

Where: Z = umber of C – atoms of the 1st standard

Z + i = Number of C – atoms of the 2nd standard

X = Substance to be calculated

Rt = Retention time

GC – MS Analysis

This was carried out on a Finnigan/Mat GC – MS instrument model 1020 B equipped with a J & W Scientific Inc. Column (30M long, 0.32mm ID, 0.25 M film coated with D8-5). Carrier gas, helium (14 psi). Injection volume 1 L split 1:20. Initial temp. 50°C programmed 4°C per minuteto final temp. 230°C. MS conditions: ionisation energy 70eV, multiplier spanning 2000V, temp. of ion source 180°C. Scan from 35 to 320 AMU in 1.0 sec. The instrument also processed the mass spectral data and carried out computerised library searches.

Analytical GC

The analytical GC used for calculation of Retention Index (RI) was measured on a varian 3700 instrument coupled to a 3390 A Hewlett Packard integrator and fitted with a J & W Scientific Inc. fused silica capillary column (30 M long, 0.25 mm ID, 0.25 M film coated with DB5 – 3N). Attenuation 2. Initial temp. 50°C programmed 4°C/min. to final temp. 230°C.

RESULTS AND DISCUSSION

Figures 1 and 2 show the capillary GC and reconstructed ion current (RIC) respectively of the oxygenated fraction of the Nsukka Yellow Pepper oil. Table 1 shows both the confirmed constituents of the oil and those indicated by interpretation of the MS of the constituents. The peak numbers in Table 1 correspond to the peak numbers of figures 1 and 2. Percentages recorded in Table 1 were calculated by the Hewlett Packard Integrator.

Components of the oil were identified by interpreting their MS, (4) comparing these with the MS and retention time of authentic compounds obtained on the same instrument and the MS of the same compound in the literature where available. Final confirmation of identity was claimed in most cases only when the retention index (RI) of the individual component of the oil also corresponded with that of the indicated authentic sample as calculated from capillary GC measured on the same instrument under the same conditions.

Strong absorptions at 1740 and 1734 cm^{-1} in the IR spectrum of the oil indicated the presence of aliphatic esters and possible aliphatic aldehydes but uniformly negative aldehydic test excluded the presence of aldehydes. This was remarkable because at least 8 aldehydes and 5 ketones were confirmed in bell peppers *(C. annuum)* (6) and 2 aldehydes but no ketones in tabasco peppers (*C. fruitescens*) (5). Also, the presence of free alcohols intermolecularly H-bonded was shown by a broad band located in the region 3600 – 3300 cm^{-1}, but a large ester value of 221 indicated the prevalence of esters over alcohols of MS that more than 91% of the oil is made up of esters while free alcohols account for only 3.8% (Peaks 2, 5, 9 and 22. Table 1). The presence of a reasonable amount of unsaturation (Peaks 5, 9, 16, 22, 23, 24, 30, 41, 42, 51, 55, 57, 58, 59, 61, 64, 67, 69, 70, 71 Table 1), in the components of the oil was shown by an iodine value of 16 but the total absence of aromatic rings was indicated by the absence of IR absorptions in the region 1600 – 1500 cm^{-1}. Here again, the difference between the constituents of the Nsukka Yellow Pepper and those of other investigated capsicums becomes apparent for the tabasco peppers *(C. fruitescens)* essential oil contains 2 oxygenated aromatics (benzaldehyde and methylsalicylate) (5) whilst bell peppers *(C. annuum)* contains 3 (benzaldehyde, phenylacetaldehyde and methylsalicylate) apart from oxygenated heteroaromatics. A strong absorption band at 970cm^{-1} due to C-H out of plane deformation and medium absorption at 720 cm^{-1} indicated that the olefinic substitutions are a mixture of both trans – and cis.

The 6 asterisked esters (Table 1) were also identified by Haymon and Aurand (5) in the volatile oil of tabasco peppers (capsicum fruitescens). Of the 41 compounds present in more than trace amounts (0.1% and above) in the highly fragrant oxygenated fraction of the Nsukka Yellow Pepper essential oil, 37 are esters and this is the highest assortment of esters so far found in the essential oil of any one member of the genus Capsicum. Hayman and Aurand (5), found a total of 18 esters in their Tabasco pepper *(C. fruitescens)*, but Buttery et al. (6) found only one ester (methyl salicylate) in the volatile oil of Bell peppers (Capsicum annuum var. grossum, sendt). The great difference in the volatile constituents of the two, (Nsukka Yellow and Bell peppers) eventhough they are both classified as *C. annuum* is to be expected because the two have quite different aroma and the difference even extends to taste for, while the Nsukka yellow is extremely pungent, the Bell pepper lacks pungency.

Apart from the 6 asterisked esters, the other 3 esters and the 4 alcohols (Table 1) are new to the genus Capsicum.

ACKNOWLEDGMENT:

A research grant from the University of Benin, a study visit grant to the University of Bonn by the German Academic Exchange Service (DAAD) and instrumental measurements in the Pharmaceutical Institute of the University of Bonn are gratefully acknowledged.

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TABLE 1

Constituents of the oxygenated fraction of the essential oil of the Nsukka Yellow Pepper

Peak number	Retention Index (RI)		Confirmed Constituents	Indicated constituents	Approximate % Abundance
	Calculated for peak	Calculated for Authentic sample			
1	811.5		* Ethanoic acid, ethylester		1.2
2	630.1	829.9	4-Methylepentan-l-ol		0.6
5	860.1		2-Hexen-l-ol (E)		0.5
9	1018.1			2-Methylhept-5-ene-l-ol	0.4
12	1105.1	1105.1	* Butanoic acid, 3-methyl -, 3-methybutylester		0.3
14	1112.5	1112.5	* Propanoic acid, 2-methyl -, 4-methylpentylester		2.0
16	1142.8		Butanoic acid, 4-hexenylester		0.7
17	1148.1	1148.1	Butanoic acid, 2-methylpentylester		0.7
20	1200.0	1200.0	* Butanoic acid, 2-methyl -, 4-methypentylester		4.1
21	1206.3	1206.6	Butanoic acid, 3-methyl -, 4-methypentylester		22.2
22	1219.7			4-methylnon-7-ene-l-ol	2.3
23	1231.4		Butanoic acid, 2-methyl -, 3-hexenylester		1.1
24	1235.4	1235.4	* Butanoic acid, 3-methyl -, 3-hexenylester		6.7
25	1241.8	1241.8	Pentanoic acid, 2-methypentylester		6.8
26	1246.5	1246.7	Propanoic acid, 2-methyl -, hyptylester		0.6
30	1293.4			Propanoic acid, 2-methyl -, 6-octenylester	3.0
32	1299.4		Butanoic acid, 2-methyl -, hyptylester		0.3
33	1308.9			Propanoic acid, 2-methyl -, 6-methypentylester	1.0
34	1314.0	1314.1	*Pentanoic acid, 4-methyl -, 4-methypentylester		1.1
35	1334.9		Butanoic acid, 2-methyl -, heptylester		0.7
36	1340.1		Butanoic acid, 3-methyl -, heptylester		2.7
37	1345.4	1345.5	Propanoic acid, 2-methyl -, octylester		0.3
38	1350.4		Pentanoic acid, 4-methyl -, hexylester		0.4
41	1381.9			Butanoic acid, 2-methyl -, 6-octenylester	4.1
42	1387.9			Pentanoic acid, 6-octenylester	10.1
43	1396.7			Pentanoic acid, 4-methylheptylester	10.7
44	1400.1			Butanoic acid, 3-methyl-, 4-methylheptylester	0.9
49	1438.7			Butanoic acid, 3-methyl-, 5-methylheptylester	1.1
51	1456.4			5-Heptenoic acid, 5-methylhexylester	0.3
55	1493.0			4-Hexenoic acid, 4-octenylester	2.5
56	1508.6			Nonanoic acid, 4-methylpentylester	0.8
57	1542.3			Hept-2, 5-dienoic acid, 5-methylhept-3-enylester	0.1
58	1580.2			Pentanoic acid, 4-methylnon-7-enylester	0.1
59	1590.9			4-Hexenoic acid, 6-methyl-2-propyl-2-heptenylester	0.5
61	1620.7			4-Hexenoic Acid, 9-methyldecylester	0.3
64	1690.8			4-Hexenoic acid, 9-methyldec-4-enylester	0.6
65	1706.8			Nonanoic acid, 8-methyl-, 4-methylpentylester	0.3
67	1727.1			4-Hexenoic acid, 9-methyldec-7-enylester	0.3
69	1868.7			Non-7-enoic acid, 4-methyl-, 2-methyloct-5-enylester	0.4
70	1886.3			Non-3, 7-dienoic acid, 4-methylhept-5-enylester	0.3
71	2065.3			Oct-6-enoic acid, 4, 4-dimethyl-, 4-methylnon-7- enylester	0.3

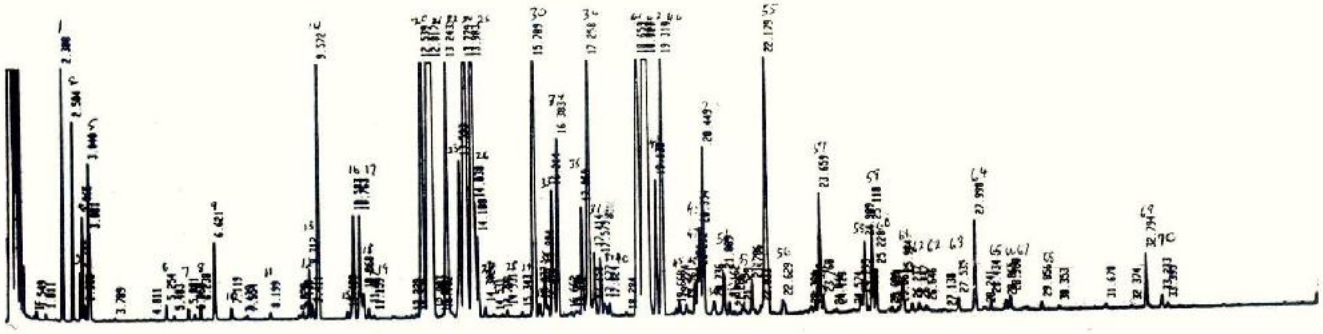

Fig 1 Capillary Gas Chromatogram of the oxygenated fraction of the essential oil of Nsukka Yellow Pepper

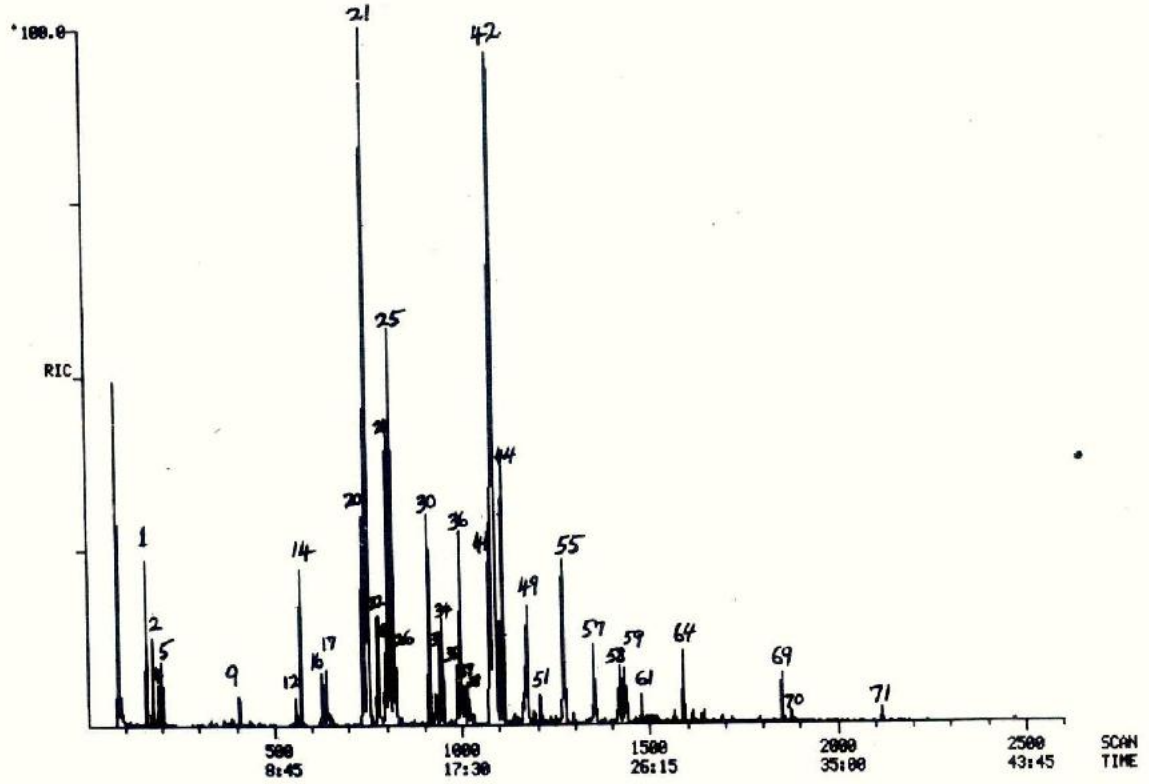

Fig 2 Reconstructed Ion Current (RIC) of the oxygenated fraction of the essential oil of Nsukka Yellow Pepper.