ESSENTIAL OIL AND LEAF CONSTITUENTS OF *LIPPIA UKAMBENSIS* FROM TANZANIA

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ABSTRACT.—Essential oil of *Lippia ukambensis* Vatke contains camphor (36.5%), 4-thujanol (18.5%), and seven other identified terpenoid substances. Extracts of leaves contained common fatty acids, stigmasterol, phytol, ursolic acid, and camphene glycol. The latter compound has not previously been reported in plants. Essential oil and leaf extracts showed no insecticidal, insect repellent, or significant antimicrobial properties.

Lippia ukambensis Vatke, of the *Verbenaceae* family, is a shrub which grows to a height of one to two meters (1). It is found mainly in long-destroyed forest sites and abandoned cultivations and is common in the Arusha region of Tanzania.

The leaves of the shrub are claimed to have a preservative effect on foods and are used by local people especially to wrap meat (2). The plant also enjoys a high reputation in the area as a remedy for various abdominal complaints (2). In view of the above and also since *L. ukambensis* Vatke has not previously been examined, it was included in the present investigation.

DISCUSSION

The composition of *L. ukambensis* Vatke essential oil is illustrated on table 1, where the techniques used for identification of components are listed. Although

TABLE 1. Volatile neutral constituents of *Lippia ukembensis* Vatke.

	Constituent	% Total	Identification method
1.	α-Pinene	0.3	ms,pe
2.	Camphene	4.0	ms,pe
3.	β-Pinene	2.1	ms,pe
4.	Monoterpene hydrocarbon (M.W.=136)	0.9	
5.	Limonene	2.2	ms,pe
6.	Cineole	11.3	ms,pe
7.	4-Thujanol	18.5	ms,pe,ir,nmr
8.	Monoterpene alcohol (M.W.=154)	2.0	
9.	Camphor	36.5	ms,ir,pe
10.	Monoterpene alcohol* (M.W.=154)	5.3	
11.	Monoterpene alcohol* (M.W.=154)	7.0	
12.	α-Terpineol	2.3	ms,pe
13.	Sesquiterpene hydrocarb (M.W.=204)	0.5	
14.	Sesquiterpene hydrocarbon (M.W.=204)	0.3	
15.	β-Cubebene	6.5	ms,pe,ir

*These components were incompletely resolved pe=peak enrichment obtained by co-injection of authentic material. Results are for two columns.

it was possible to definitely identify only nine of the fifteen observed compounds, these account for 84% of the total material. The unidentified components consisted of a monoterpene hydrocarbon (0.9%), three monoterpene alcohols (two

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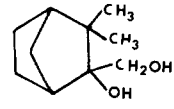
incompletely resolved) (14.3%), and two sesquiterpene hydrocarbons (0.8%). The oil is rich in camphor and also contains substantial amounts of 4-thujanol.

Tests on the oil showed that it did not possess any insecticidal or insect repellent properties (mosquitos), and it displayed antimicrobial activity against only one of the four organisms used (*S. Aureus*). Its minimum inhibitory concentration was 800 μg/ml for this organism.

Solvent extracts of the leaves of the plant contained common fatty acids and their ethyl esters (artefacts formed during extraction), stigmasterol, phytol, ursolic acid and camphene glycol (3). The discovery of the latter compound is worthy of note since it has not previously been found in plants.



Camphene glycol was isolated in the form of colorless plates, mp 180–181°, and its molecular formula $C_{10}H_{18}O_2$ was confirmed by element analysis and mass spectrometry. The nmr spectrum showed two singlet methyl groups and one downfield singlet methylene group together with two exchangeable protons. The mass spectrum had peaks indicating loss of H_2O (152) and loss of $-CH_2OH$ (139) in accord with the above structure. For confirmation of the structure authentic camphene glycol was prepared by hydroxylation of camphene with osmium tetroxide (4). The synthetic compound had ir, nmr and mass spectra data identical to the compound isolated from the plant and also gave spots on tlc plates with the same Rf values, but there was a discrepancy in the melting point which was 193–194°. It is likely that the naturally occurring material is optically active, whilst the synthetic compound has been reported to be a racemate (5) which can account for the difference in melting points. The amount of glycol isolated represented 0.007% of the weight of fresh leaves.

Biological tests did not explain the use of leaves of *L. ukambensis* for food preservation nor the plants' medicinal effects. Neither the essential oil nor plant extracts possessed any insecticidal, larvicidal, or insect repellent properties. Anti-fungal and anti-bacterial properties were also lacking. The medicinal effects claimed may, however, be due to the combination of ingredients in the essential oil, especially camphor. No alkaloids were detected in the plant.

EXPERIMENTAL[2]

PLANT MATERIAL.—The plant material, *L. ukambensis* Vatke, was collected at Arusha and Lushoto, Tanzania, in the month of January. Identification was made by the Herberium of the University of Dar es Salaam and the Herberium of the Tropical Pesticide Research Institute at Arusha. A specimen of the plant was deposited at the University of Dar es Salaam as J. B. Chogo 3.

PLANT DISTILLATION.—Freshly picked leaves, when steam distilled in a conventional still, yielded an essential oil (0.3%), a pale yellow liquid, d_{20}^{20}, 0.923; $n^{20}D$, 1.4754; $[\alpha]^{20}D$, −29.9; solubility 1:1.5 in 70% ethanol.

PLANT EXTRACTION.—Leaf components were obtained by extracting the fresh plants with 95% ethanol at ambient temperature. The crude extract was filtered and concentrated at <40° in a rotary evaporator. The residue was re-extracted with diethyl ether and was then separated into neutral, basic (extracted by 10% HCl solution), and acidic (extracted by 10% NaOH solution) fractions.

[2]The oil was analyzed by glc on a Packard model 417 instrument fitted with glass columns (6 ft x ¼″) packed with either SE30 (10%) or FFAP (5%) on Chromosorb W (80–100 mesh). Nitrogen was used as the carrier gas (60 ml/min) with temperature programming from 80–200° at 8°/min. Glc/ms analysis was performed on a Shimadzu GC 6AMP instrument with a 40M OV–17 Scot column interfaced with an AEI MS12 mass spectrometer. Helium was used as the carrier gas (2 ml/min), and the column was programmed from 55–200° at 5°/min. Preparative glc was performed on a Varian 712 instrument fitted with a 20% Carbowax 20M column (12 ft x ⅜″), programmed from 100–200° at 2°/min with a flow rate of 100 ml/min of nitrogen. A Pye-Unican SP1000 was used for ir spectra, and a Jeol NM4H was used for nmr spectra.

CHROMATOGRAPHY OF ACIDIC MATERIAL.—The acidic fraction (3 g from 15 g crude extract) was separated on a column (7 cm ID) of Merck type 60 silica gel (150 g) packed as a slurry in light petroleum ether (bp 40–60°). Material was eluted by light petroleum ether (bp 40–60°) containing increasing amounts of ethyl acetate (5→20%). Portions (200 ml) were collected and, after examination by tlc, were bulked as appropriate.

Fractions 1–23 (1.2 g). This was a waxy residue which was shown to be mainly palmitic acid (90%) and linoleic acid (10%) by glc analysis of the prepared methyl esters.

Fractions 24–53 (0.8 g). This material was identified as a mixture of fatty acids, palmitic (48%), linolenic (39%), linoleic (8%) and others (10%), by glc analysis of methylated material.

Fractions 54–67 (0.2 g). This fraction gave colorless needles mp 266–270°. Found, C, 79.1; H, 10.4. Calculated for $C_{30}H_{48}O_3$ C, 78.9; H, 10.6%. The mass spectrum (M^+ 456), the ir and nmr spectra were identical with those of an authentic sample of ursolic acid (lit mp 285°). (6)

Fractions 68–95 (0.3 g). This fraction yielded a gummy green residue containing mainly ursolic acid plus two minor components which could not be isolated by crystallization or resolved on a preparative chromatographic plate developed with petroleum ether-ethyl acetate-ethanol (5:4:1).

CHROMATOGRAPHY OF NEUTRAL MATERIAL.—The neutral fraction (5 g from 15 g of crude extract) was similarly separated and gave the following material when the column was eluted with petroleum ether containing increasing amounts (0.25→20%) of ethyl acetate.

Fractions 1–3 (.35 g). This fraction yielded a light oil shown by glc and spectral data to be mainly β-cubebene.

Fractions 4–5 (3.0 g). An oily solid was obtained which was shown by glc analysis to be a mixture of ethyl palmitate (40%), ethyl linolenate (40%), and other ethyl esters of fatty acids (20%). Authentic ethyl esters of fatty acids were prepared and used as reference materials for glc work on two columns.

Fractions 6–20 (0.7 g). A yellow oil was isolated and was shown by tlc to contain only one component. The mass spectrum indicated a parent ion M^+ 296. The ir and nmr spectra were identical with those of authentic phytol. All tlc data of authentic and isolated material agreed.

Fractions 21–46 (0.5 g). Colorless needles were isolated with mp 169–170°. Found, C, 84.6; H, 11.5%. Stigmasterol, $C_{29}H_{48}O$ requires C, 84.5; H, 11.7% (lit mp 170°) (7). The ir and nmr spectra of isolated and authentic materials were identical.

Fractions 47–55 (0.29 g). Colorless needles were obtained by sublimation of the crude material in vacuo at 60°; mp 180–182°. Found C, 70.7; H, 10.4; $C_{10}H_{18}O_2$ requires C, 70.6; H, 10.6%; mass spectrum, *m/e* (%) 170(19), 153(2), 152(7), 140(10), 139(100), 137(11), 121(26), 111(54), 109(28), 102(16), 101(66), 96(24), 95(42), 93(23), 87(74), 85(16), 84(17), 83(17), 81(27), 79(19), 71(23), 70(13), 69(84), 67(50), 56(11), 55(42), 53(11), 45(10), 43(77), 41(60); ν_{max} 600, 650, 700, 725, 760, 830, 900, 955, 1040, 1100, 1150, 1210, 1240, 1300, 1380, 1470, 2950, 3400 cm^{-1}; nmr (100 Mz, $CDCl_3$), δ 1.0 (d, 6H, 2xCH_3), 1.83 (broad, 2H, exchanged by D_2O), 3.63 (s, 2H, CH_2 adj to OH), other peaks were unresolved. The above data fits the structure of camphene glycol.

To prepare authentic camphene glycol, camphene (0.24 g) and osmium tetroxide (1 g) were stirred in dry cyclohexane for 1 hr at 20°C (4). The mixture was filtered, and the precipitate (1.13 g) was then dissolved in a solution (30 ml) of potassium hydroxide (25%) containing mannitol (7.5%). The solution was warmed to 60° for 15 mins, cooled and continuously extracted with methylene chloride for 12 hrs. Concentration of this extract gave an off-white solid (0.41 g—62%) purified by vacuum sublimation. Camphene glycol formed colorless plates, mp 193–194° (Lit mp 194–196°) (5). Found C, 70.9; H, 10.6. Calculated for $C_{10}H_{18}O_2$, C, 70.6; H, 10.6%.

CHROMATOGRAPHY OF THE BASIC FRACTION.—The basic fraction (0.14 g from 15 g crude extract) was examined by tlc with methanol/ammonia as solvent (100:1). Plates were sprayed with Marquis, Dragendorf and iodoplatinate reagents with entirely negative results for alkaloids.

BIOLOGICAL ACTIVITY TESTS.—Insecticidal tests were carried out on *Musca domestica*, larvicidal and insect repellent tests on *Aedes aegypti* and anti-microbial tests on *Escherichia coli*, UNSW 024101, *Micrococcus luteus* ATCC 9341, *Staphylococcus aureus* ATCC 6538 and *Saccharomyces cerevisiae* UNSW Y-40. The testing methods are as described in our previous paper (8).

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