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VARIETAL DIFFERENCES IN THE CONSTITUENTS OF CITRONELLA OIL

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Abstract—Gas chromatography of citronella oil showed that the Ceylon variety (Lenabatu) contained large amounts of monoterpene hydrocarbons, while the Java variety (Mahapengiri) contained only small amounts, mainly limonene. Both types contained comparable amounts of geraniol, and the Java type more of citronellol and citronellal. In addition the Ceylon type contained tricyclene, methyl eugenol, methyl isoeugenol, eugenol and L-borneol. The GLC profiles enable the identification of the type of oil and the detection of kerosene as a possible adulterant. The variety that grows wild in Ceylon (Mana) was quite different to both cultivated types.

INTRODUCTION

CITRONELLA oil is the essential oil from citronella grass. Two closely related types of citronella grass are cultivated on a large scale.[1] They can be distinguished morphologically by the shape and length of their leaves, and chemically,[2–4] by the composition of the essential oil obtained from them. Joachim and Pandittasekera[5,6] have shown that both varieties are responsive to fertilizer application. The yield of oil can be increased four-fold in 2 yr. Botanically the two types Lenabatu and Mahapengiri are derived from *Cymbopogon nardus* (L.) Rendle (= *Andropogon nardus Ceylon* de Jong) *Cymbopogon winterianus* Jowitt (= *Andropogon nardus Java* de Jong) respectively.[7] It is believed[3,4] that both types originated in Ceylon. Lenabatu is the type now mostly cultivated in the island, and Mahapengiri is grown mainly in Java, Haiti, Honduras, Taiwan, Guatamala, and the People's Republic of China.

A type which is probably the parent of the cultivated citronella grasses grows wild in Ceylon[1,3] and several varieties of this are known under the common name of 'mana grass'. The oil from these varieties are not commercially exploited as the 'total acetylisables' in them amount to only about 20%. Two of these wild varieties are mentioned in the literature[2,3] namely *C. nardus* var Linnaei (typicus) and *C. nardus* var *confertiflorus* but there appears to be many more than these.[8]

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[1] SENARATNE, J. E. (1956) *The Grasses of Ceylon*, Peradeniya Manual No. 8, p. 193, Government Press Colombo, Ceylon.

[2] JOWITT, J. F. (1908) *The Volatile Oils* (GILDEMEISTER, A. and HOFFMANN, A. eds., KREMERE, E., transl.), 2nd Edn, Vol. 2, pp. 217–243, *Annals Royal Botanic Gardens, Peradeniya* **41**, 185.

[3] GUENTHER, E. (1950) *The Essential Oils*, Vol. 4, p. 67, Van Nostrand, New York.

[4] GUENTHER, E. (1940) *Soap Sanitary Chem.* **16** (10), 32, 73.

[5] JOACHIM, A. W. R. (1929) *Trop. Agric.* **73**, 136.

[6] JOACHIM, A. W. R. and PANDITTASEKERA, D. G. (1953) *Trop. Agric.* **109**, 1.

[7] ABEYWICKREMA, B. A. (1959) *Ceylon J. Sci.* (*Bio. Sci.*) **2**(2), 132.

[8] WIJESEKERA, R. O. B., PONNUCHAMY, S. and JAYEWARDENE, A. L. (1972) *Proc. Ceylon Assoc. Advanc. Sci.* **28**, 118.
RAJAMANI, T. S., RAMACHANDRA D., RANGE GOWDA, RAO, D. and RAMASAMY, M. N. (1965) *Perf. Essent. Oil Rec.* 726.

The early efforts at identifying the chemical constituents of citronella oil[3,9] were dependent on comparatively drastic fractional distillation procedures, and many of the minor components could well have been artefacts formed on heating. The main chemical differences hitherto recognized between the oil from the two varieties are in the relative amounts of acetylisable compounds present in them,* and the occurrence of compounds related to eugenol in the Lenabatu variety. Recently, Rogers,[10] Rogers and Toth[11] and Humphrey[12] have conducted studies on the two types of citronella oil using GLC methods. Rogers and Toth[11] reveal that the two types of oil differ more markedly in their citronellol content than in the amount of geraniol present. In addition these workers and Ikeda *et al.*[13] have found in the Ceylon citronella a higher percentage of monoterpene hydrocarbons.

There are striking differences between the citronella oil from the two major types, Lenabatu, and Mahapengiri and these differences may be useful in chemotaxonomy. We have also studied the oil from the 'wild' varieties of citronella, known in Ceylon under the collective name of 'mana grass'.[1–4] However, while we have examined the oil of freshly distilled Lenabatu and Mana we have not studied freshly distilled samples of the oil from the Java variety Mahapengiri, since our samples were from commercial sources, and it is not known whether they had been subjected to any deterpenation procedures. However, the Ceylon oil purchased from the same sources showed the same characteristics as those distilled by us.

RESULTS AND DISCUSSION

Typical chromatograms of the varieties are shown in Fig. 1. Generally speaking LAC-2R-446 and Carbowax 20 M gave the best resolution, as many as 60 peaks being obtained for the Ceylon variety. Peak enrichment was carried out using three stationary phases. Peak

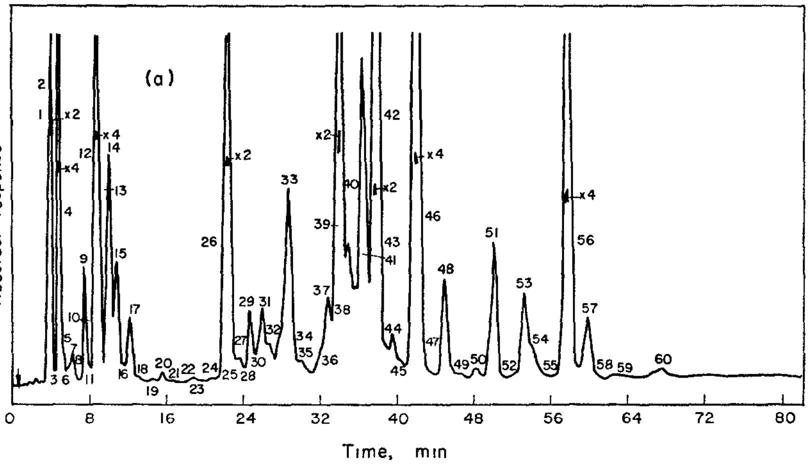

FIG. 1 (a). GAS CHROMATOGRAM OF LENABATU OIL (CEYLON TYPE) CITRONELLA.

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* Referred to in commercial circles as the 'geraniol content' or 'total acetylisables expressed as geraniol'.

[9] GUENTHER, E. (1968) *Am. Perf. Cosmet.* **83**, 57.

[10] ROGERS, J. A. (1960) *Proc. Sci. Sect. Toilet Goods Assoc.* **32**, 9.

[11] ROGERS, J. A. and TOTH, Z. E. (1961) *Proc. Sci Sect. Toilet Goods Assoc.* **35**, 29.

[12] HUMPHREY, A. M. (1970) *J. Chromatog.* **53**, 375.

[13] IKEDA, R. M., STANLEY, W. L., VANNIER, S. H. and SPITLER, E. M. (1962) *J. Food Sci.* **27**, 455.

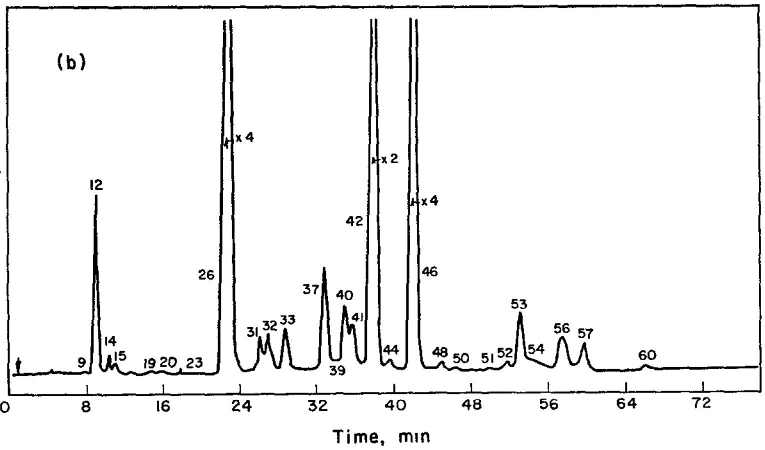

FIG. 1 (b). GAS CHROMATOGRAM OF MAHAPENGIRI OIL (JAVA TYPE) CITRONELLA.

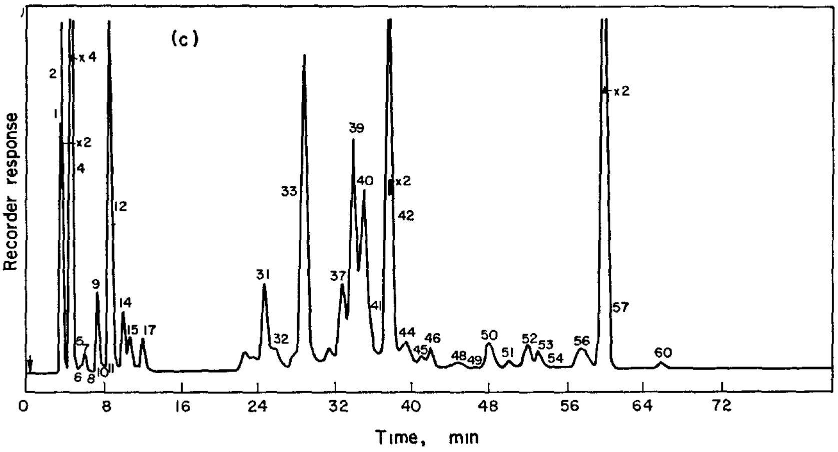

FIG. 1 (c). GAS CHROMATOGRAM OF MANA OIL (WILD TYPE).
For conditions see Experimental. The stationary phase used was Carbowax 20 M.

identifications are recorded in Table 1. The identifications were based on IR spectra, retention data,[14] peak enrichment, TLC and in some cases chemical evidence depending on whether they underwent silylation or acetylation. The Ceylon oil had a high proportion (about 27 %) of monoterpene hydrocarbons, as against 1–3 % (mainly limonene) in the Java variety.

Of the hydrocarbons in the Lenabatu type* camphene was the most abundant, while the

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* Commercial Ceylon oil is distilled mainly from the Lenabatu type of grass but there are areas growing the Mahapengiri-type as well. Some Mahapengiri-type oil invariably enters the oil produced commercially. We have observed[8] that even in the oil of selected Mahapengiri-types grown in Ceylon, there is a high distribution of terpene hydrocarbons though not to the same extent as in Lenabatu. This may indicate that these Mahapengiri varieties are hybrids.

[14] AMBROSE, D., KEULEMANS, A. I. M. and PURNELL, L. H. (1958) *Anal. Chem.* **30**, 1582.

TABLE 1. COMPARISON OF COMPONENTS OF MAHAPENGIRI AND LENABATU CITRONELLA OIL

Peak No.	Compound	Methods of identification†	Approximate percentage present in citronella oil	
			Mahapengiri	Lenabatu
1	Tricyclene	RD, PE, IR,	—	1·6
2	α-Pinene	RD, PE, IR,	—	2·6
3	—	—	—	—
4	Camphene	RD, PE, IR,	—	8·0
5	β-Pinene	RD, PE,	—	Trace
6	Sabinene	RD, PE,	—	Trace
7	Myrcene	RD, PE, IR,	—	0·3
8	Car-3-ene	RD, PE,	—	Trace
9	α-Phellandrene	RD, PE,	—	0·8
10	α-Terpinene	RD, PE,	—	—
11	—	—	—	—
12	Limonene	RD, PE, IR,	1·3	9·7
13	—	—	—	—
14	*cis*-Ocimene; γ-Terpinene	RD, PE,	—	1·4
15	*trans*-Ocimene; β-Phellandrene	RD, PE,	—	1·8
16	*p*-Cymene	RD, PE,	—	Trace
17	Terpinolene	RD, PE,	—	0·7
18	—	—	—	—
19	—	—	—	—
20	1-Hexanol	RD, PE,	—	0·1
21	—	—	—	—
22	—	—	—	—
23	Methyl heptenone	RD, PE,	Trace	0·2
24	—	—	—	Trace
25	—	—	—	Trace
26	Citronellal	RD, PE, Ch, TLC IR,	32·7	5·2
27	Camphor	RD, PE, Ch,	—	0·5
28	Bourbonene	RD, PE, Ch,	Trace	1·0
29	Linalool	RD, PE, Ch, TLC, IR,	1·5	1·2
30	Linalyl acetate	RD, PE, IR,	2·0	0·8
31	—	—	—	—
32	α-Terpeneol	RD, PE, IR,	—	Trace
33	β-Caryophyllene	RD, PE, Ch, IR,	2·1	3·2
34	4-Terpinenol	RD, PE, Ch	Trace	0·7
35	Menthol	RD, PE, Ch	—	Trace
36	—	—	Trace	Trace
37	Citronellyl acetate	RD, PE,	3·0	1·9
38	—	—	—	Trace
39	L-Borneol	RD, PE, IR, MS, NMR,	Trace	6·6
40	Geranyl formate	RD, PE,	2·5	4·2
41	—*	—	—	—
42	Citronellol; Geranyl acetate*	RD, PE, Ch, TLC, IR, (both)	15·9	8·4
43	—	—	—	—
44	Nerol	RD, PE, Ch,	7·7	0·9
45	—	—	—	—
46	Geraniol	RD, PE, Ch, TLC, IR,	23·9	18·0
47	Citronellyl butyrate	RD, PE	Trace	Trace

TABLE 1—*Continued*

Peak No.	Compound	Methods of identification†	Approximate percentage present in citronella oil Mahapengiri	Lenabatu
48	Geranyl butyrate	RD, PE	—	1·5
49	—	—	—	—
50	Nerolidol	RD, PE,	—	0·3
51	Methyl eugenol	RD, PE, Ch, TLC, IR,	Trace	1·7
52	—	—	—	—
53	Elemol	RD, PE, IR,	6·0	1·7
54	—	—	—	—
55	—	—	—	—
56	Methyl *iso*-eugenol eugenol	RD, PE, Ch, TLC, IR,	2·3	7·2
57	—	—	1·4	1·5
58	—	—	—	—
59	—	—	—	—
60	Farnesol	RD, PE,	0·6	Trace

* Minor component which merges into major peak; separate peaks are revealed at optimum loading of column.

† *Abbreviations*: RD—Retention data; PE—Peak enrichment technique; Ch—Chemical methods: silylation, hydrolysis, etc.; TLC—Thin layer chromatography; reaction of spraying with Vanillin-H_2SO_4 reagent; IR—Infra-red spectrum and its identity with a spectrum of the authentic compound; MS—Mass spectral data; NMR—Nuclear magnetic resonance data.

presence of the solid tricyclic hydrocarbon tricyclene is reported here for the first time. Other hydrocarbons present are α-pinene, β-pinene, sabinene, myrcene, car-3-ene, α-phellandrene, α-terpinene, limonene, *p*-cymene, *cis*- and *trans*-ocimene, γ-terpinene, β-phellandrene and terpinolene.

The frequent co-occurrence of tricyclene and camphene in essential oils has been observed by Zavarin.[15] This phenomenon could be attributed to the fact that both these compounds are products of the same biosynthetic pathway from geranylpyrophosphate via carbonium ions.[15,16]

The occurrence of L-borneol, bornyl acetate, and a trace of camphor in the Ceylon oil is also compatible with this scheme, as the 2-bornane-carbonium ion can give rise to both borneol and camphor. Zavarin and Snajberk[17] have previously noted the co-occurrence of these compounds together with camphene and tricyclene in *Abies* cortical oleoresin. It has frequently been recorded[3,4] that the commercial citronella oil from Ceylon is adulterated with mineral oils such as kerosene. This may well have been so in the past, but the evidence for this is larely based on arbitrary tests such as 'Schimmels Test',[3] 'Raised Schimmel's test'[3] and the 'London solubility test'.[18] The presence of kerosene hydrocarbons in citronella oil can be detected by the appearance in the gas-chromatogram of several distinctly alien peaks. Kerosene itself being a complex mixture of hydrocarbons, gives rise to a great number of peaks many of which merge into the peaks corresponding to the authentic

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[15] ZAVARIN, E. (1970) *Phytochemistry* **9**, 1049.

[16] WIJESEKERA, R. O. B. (1973) *Proc. Ceylon Assoc. Advanc. Sci.* in press.

[17] ZAVARIN, E. and SNAJBERK, K. (1965) *Phytochemistry* **4**, 141.

[18] GUENTHER, E (1950) *Essential Oils*, Vol. 4, p. 78, Van Nostrand, New York; (1945) *Analyst* **70**, 442.

constituents of citronella and it is therefore difficult to detect the kerosene. Three of the main peaks of kerosene, however, fall into the region occupied by the oxygenated constituents of citronella. Removal of the oxygenated compounds by adsorption on silica gel, greatly facilitates the detection of these peaks (Fig. 2). In the several commercial oils that we have recently examined we have detected no kerosene. It has been previously observed by Joachim[5] that even freshly distilled oils of the Ceylon variety often failed to pass the accepted 'solubility tests'. We too have found that oils distilled by us from fresh authentic citronella grasses failed to pass these tests. It is possible that the presence of a large percentage of terpene hydrocarbons in the Ceylon oil is responsible for this situation, so that these tests, which are based on mere solubility characteristics are not a reliable guide to the authenticity of the oil.

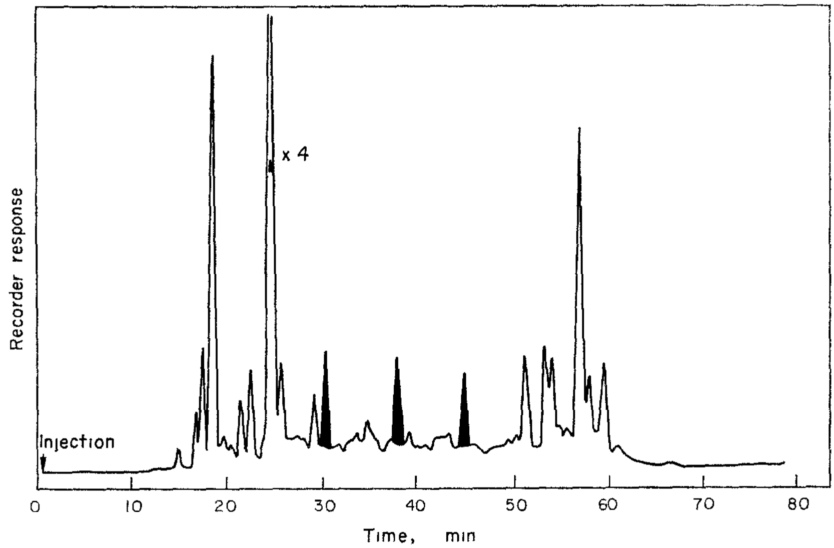

FIG. 2. GAS CHROMATOGRAM OF CITRONELLA OIL (CONTAINING ABOUT 5% KEROSENE ADULTERANT) RECORDED AFTER REMOVAL OF THE OXYGENATED COMPOUNDS BY ADSORPTION ON SILICA GEL. THE KEROSENE PEAKS ARE SHADED.
(For conditions see Experimental).

The Java oil contains a higher content of oxygenated constituents. The quantity of geraniol in both types is of the same order, but the Java variety contains a higher proportion of both citronellal and citronellol. This accounts for its higher content of 'total acetylisables' by which criterion it is often preferred to the Ceylon oil in commerce, although the Ceylon oil is used in soap.

Our results here are in accord with those of Rogers.[10] As mentioned earlier the Ceylon oil contains l-borneol (peak 39), a solid, which can be readily isolated by fractional distillation.[3] The Java oil has only a trace of this substance. The occurrence of elemol has also been recorded before[3] and we have confirmed its presence in Ceylon citronella oil. However, Sutherland and Jones[19] have recently suggested that elemol may be formed as an artefact, and that the authentic natural product may be its thermolabile precursor hedycaryol.

[19] JONES, R. V. H. and SUTHERLAND, M. D. (1968) *Chem. Commun* 1229.

In order to verify this hypothesis in the case of citronella we chromatographed the oil obtained by cold percolation of the macerated fresh grass according to the method of Southwell[20] using a preparative column and a low injection temperature (90°). The column temperature was isothermal at 80° over a very long period of time. Several compounds were collected and the IR spectrum of one of these agreed almost exactly with the spectrum of authentic hedycaryol kindly sent to us by Dr. M. D. Sutherland. The presence of hedycaryol in Ceylon citronella oil has since been confirmed.[21] One of the most significant differences between the Java type of oil and the Ceylon type is the presence in the latter of the phenolic derivatives methyl eugenol and methyl isoeugenol (peaks 51, 56). The Java variety has only traces of these compounds, while some of the wild-varieties of citronella growing in Ceylon contain them in abundance.

The presence of cadinenes have been reported[11] as well as several trace amounts of sesquiterpenes[9] in Java citronella oil. We were unable to assign any peaks to the cadinenes although our resolution was better than that of Rogers[11] who reported the presence of these. We are unable to explain the absence of these compounds in our chromatograms and lack of authentic compounds prevents us from examining this further. We however, obtained a significant peak (peak 57) in the Ceylon oils which is probably a hydrocarbon. This may be due to sesquicitronellene which has been reported previously.[3]

EXPERIMENTAL

Source of materials. Samples of commercial citronella oil, both Ceylon and Java varieties, were obtained from commercial sources. Samples of the Ceylon variety were also obtained from Messrs. Volanka (Ceylon) Ltd., and Mr. T. P. G. Atukorale, Colombo Commercial Company. In addition, oils of the local varieties were also distilled from grasses gathered by Mr. S. Ponnuchamy directly from the growing areas. The steam distillations were carried out under standard conditions, and also in the field.[16]

Gas chromatographic parameters. (a) *Analytical GLC of citronella oils.* This was achieved on a Varian Aerograph Moduline (1740–2) with F.I.D. The following columns were employed: (1) Carbowax 20 M 10% on Chromosorb W (2·7 m × 3·2) mm. (2) LAC-2R-446, 5% on Firebrick (3 m × 3·2 mm). (3) SE30, 15% on Chromosorb W (3 m × 3·2 mm). Injection block temp., 200°; detector oven temp., 230°, carrier gas (argon) 25 ml/min. Samples (0·2 μl), with programming from 60 to 200° (190° for packing (2) at 2°/min. (b) *Preparative GLC of citronella oils.* This was achieved on a Varian Aerograph (model 90 P3) with thermal conductivity detector (150 mA) columns. (1) Carbowax 20 M, 10% on Chromosorb W (3 m × 6·4 mm) (2) LAC-2R-446, 15% on Firebrick (3 m × 9·5 mm) (3) FFAP, 15% on Chromosorb W (3 m × 6·4 mm). Injection block temp. 200°; detector oven temp. 220°. Carrier gas (helium) 50 ml/min. Samples (20–100 μl), with programming from 60 to 220° at 2°/min. (c) *Detection of kerosene.* This was achieved on a Varian Aerograph Moduline (1740–1) with F.I.D. Column packing SE 30 on Chromosorb W (3 m × 3·2 mm). Injection block temp. 200°; detector block temp. 260°; Carrier gas (argon) 25 ml/min. Samples (0·05 μl), Argon 25 ml/min with programming from 60 to 220° at 2°/min. Quantitative estimation of the constituents was carried out by determining the peak areas by triangulation. To ensure precision all peak measurements were made as reported earlier.[22,23]

IR spectra. IR were run as smears or as solutions in $CHCl_3$ (using $CHCl_3$ as reference) with NaCl optics in a Perkin–Elmer 700, double beam spectrophotometer.

Identification of constituents. Tentative peak identifications were assigned by comparing the corrected retention vols with those of authentic compounds,[14] using columns with the three different stationary phases. The techniques of peak enrichment were employed in all four columns to confirm identification.[22,23] Chemical techniques were combined with GLC for further confirmation of the identifications. Firstly the oil was acetylated and it was observed that the peaks suspected to be compounds which were acetylisable disappeared from their normal position, and appeared as the acetyl-derivatives. Silylation was also used in this fashion, to confirm the identifications. The monoterpene and sesquiterpene hydrocarbons were confirmed in this manner as they persisted after this treatment. Several of the oxy-derivatives were confirmed by collection from the

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[20] SOUTHWELL, I. A. (1970) *Phytochemistry* **9**, 2243.

[21] SUTHERLAND, M. D. private communication.

[22] BERNHARD, R. A. (1962) *Anal. Chem.* **34**, 1476.

[23] BERNHARD, R. A., WIJESEKERA, R. O. B. and CHICHESTER, C. O. (1971) *Phytochemistry* **10**, 177.

preparative column according to the methods described by Jennings *et al*,[24] and also by bubbling the GLC effluent into $CHCl_3$-cooled to −50°. Compounds obtained in this fashion were subjected to analysis by TLC, their colour response to the vanillin-H_2SO_4 reagent, and IR spectroscopy, the spectra being compared with published spectra and/or spectra of authentic compounds In order to facilitate the collection of compounds by preparative GLC the oil was prefractionated by distillation under vac. (10 mm Hg) into three fractions. Fraction 1, b.p. range 80°; Fraction 2, b.p range 80–100°; Fraction 3, b p. range 110°, Fraction 4, b.p. range 130°. These fractions were used in preparative gas chromatography

Detection of kerosene. Kerosene oil (from the Sri Lanka Petroleum Corporation) was added to freshly distilled citronella oil up to 5% by vol To this mixture (1 ml) was added pure hexane (80 ml) and silica gel (20 g) which had been previously heated at 130° for 2 hr. After standing overnight the supernatant was filtered and most of the hexane removed by distillation under vac. The remaining soln was examined by GLC and the chromatogram compared with one of pure citronella oil which had been similarly treated. Three easily recognisable peaks are attributable to kerosene (Fig 2).

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[24] JENNINGS, W. G., CREVELING, R. K. and HEINZ, D. E. (1964) *J. Food Sci.* **29**, 730.