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The Chemical Composition and Analysis of Citronella Oil*

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1. *Introduction*

Citronella oil is the essential oil obtained from citronella grass which is grown predominantly in the south of Sri Lanka. The two cultivated types are known as MAHAPENGIRI and LENABATU and they are distinguished morphologically by the shape and length of their leaves[13]. The two types are considered to be derived from the same parent ; and are now botanically classified as [1,5]:—

Lenabatu	(= Heen pengiri)	:	*Cymbopogon nardus* (L) Rendle
	(Ceylon type)	=	*Andropogon nardus* Ceylon de Jong
Mahapengiri	(Java type)	:	*Cymbopogon winterianus* Jowitt
		=	*Andropogon nardus* Java de Jong

Differences in the chemical composition of the essential oils from the two varieties have been recorded since early times[5,9]. It was believed that the Mahapengiri variety contained around 85% of "total acetylisables expressed as geraniol" the chief among which were geraniol (I), citronellal (II) and citronellol (III). The Lenabatu variety on the other hand was reported to contain only 55—65% of "total acetylisables expressed as geraniol". Both types of oil were, and still are, in demand in commerce. The Lenabatu-type is used as a deodorant in mosquito repellent applications, and for the scenting of soaps, sprays, disinfectants, paints and polishes. The Mahapengiri-type is used mainly as a starting material for the preparation of industrially important perfumery compounds derived from geraniol, citronellol and citronellal, for which purpose the Lenabatu-type is not as suitable.

* Based on a lecture delivered at the Institute of Chemistry, Ceylon, *Annual Sessions*, June 1973.

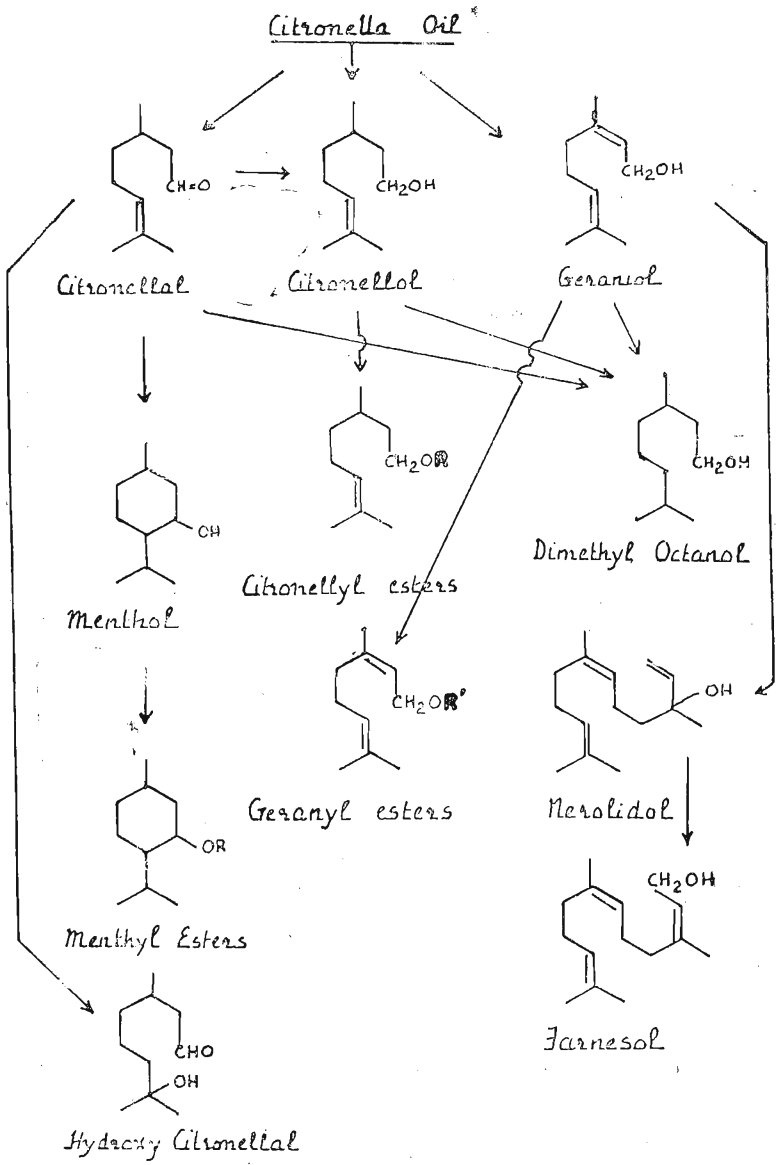


Chart 1. Synthetic perfumery compounds obtainable from Citronella Oil.

The main constituents of the two types of citronella oil had been "identified"[5] by means of classical chemical methods (*Table* 1). These classical methods were based on comparatively drastic fractional distillation procedures. Hence

it was quite possible that chemical transformations took place and some at least of the products were artefacts not present in the original oil. Yet the identification and characterisation of these compounds at the time, were laudable feats of chemistry[14]. The classical methods of analysis[5] of the essential oil of citronella were primarily based on two factors : firstly, the estimation of the "total acetylisables" in them and secondly, various rough solubility checks known as, "Schimmel's test", "Raised Schimmel's test" and "London solubility test"[5]. In addition, the limiting values for various physical constants such as refractive index and optical rotation were specified.[3]

Table 1[5] Chemical Constituents of Citronella oil (Classical Methods).

Lenabatu	Mahapengiri	
Camphene	Limonene	Citronellyl oxide
Dipentene (limonene)	Citronellal	γ and δ cadinene
Citronellal	Citral	Vanillin
Geraniol	Geraniol	Isovaleraldehyde
Geranyl acetate	Citronellol	Hexene-2-al
Nerol	Geranyl butyrate	3-Methyl-pentanal
Citronellol	Citronellyl citronellate	
Thujyl alcohol	Eugenol	
Borneol	Methyl eugenol	
Farnesol	Chavicol	
Linalool	Sesquicitronellene	
Methyl eugenol	Elemol	

2. *The Advent of Instrumental Methods*

Two factors were mainly responsible for what may be called the "second look" at the volatile essential oils in general, from the view point of their chemical constituents. The first was a remarkable phase in the development of organic chemistry itself. The new techniques for the characterisation of chemical compounds based on spectroscopic methods resulted in a major surge in natural products research in general, during the 1960—70 decade[4]. These techniques not only afforded a deeper insight into structural features of

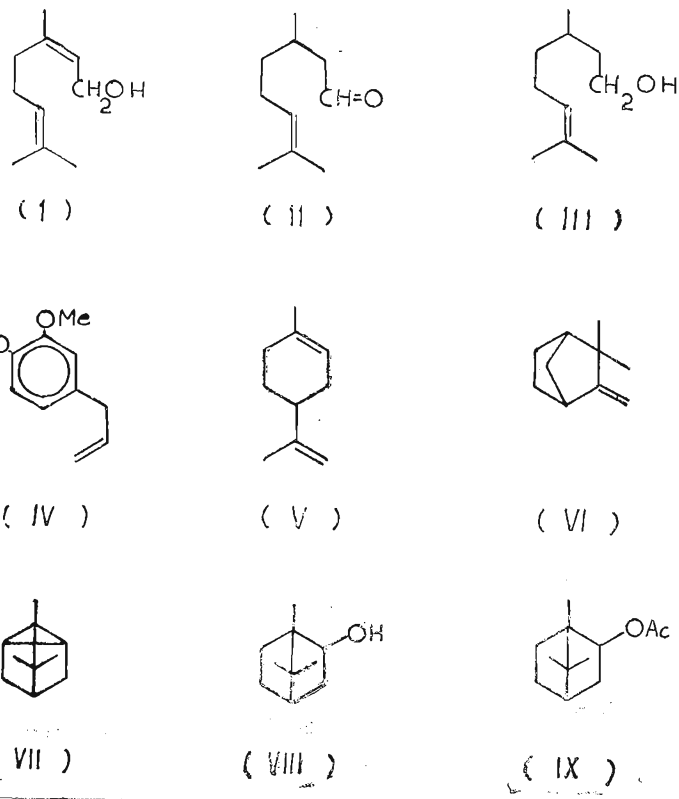
organic molecules, but they also needed comparatively small quantities of compounds. Prior to this, the characterisation of organic compounds was dependent on several time-consuming operations ; firstly the preparation of derivatives and determination of their physical constants such as melting point, optical rotation, etc ; then the degradation or conversion to known compounds; and finally identifying the inter-relationship between the degradation products and the original whole - an exercise which was much in the nature of piecing together a complex jigsaw puzzle. These operations needed comparatively large amounts of material which were often difficult to obtain. The second factor that facilitated a re-study of essential oils was the development of new separation techniques based on chromatography. The methods of separation available previously were the comparatively drastic ones of fractional distillation and chemical reactions based on particular functional groups. Fractional distillation often caused changes due to isomerisation, polymerisation or decomposition even when carried out at reduced pressures. Separations based on chemical reactions were applicable only in comparatively few instances ; as for example, the isolation of eugenol (IV) from the other constituents of cinnamon leaf oil and clove oil, by alkali treatment. The most significant advance as far as essential oils were concerned was the development of gas-liquid chromatography (GLC). This gave a new dimension to studies on essential oils and their chemical constituents. Here was a technique that seemed from the beginning to be ideally suited to the study of essential oils. It depended much for its effectiveness on the volatility of the compounds ; and the constituents of essential oils by the very nature of their preparation are that. There were in most cases a large number of chemical compounds in essential oils, and their examination depended on the extent to which these could be effectively separated. GLC afforded a fantastic method of separation which could be achieved with very minute amounts of material. Furthermore, preparative GLC afforded a means of isolation of the separated constituents which could then be subjected to the scrutiny of new techniques, such as ir, NMR spectroscopy and mass spectrometry, or to microchemical reactions, in order to determine or ascertain their chemical nature.

The combination of GLC and infra-red (ir) spectroscopy seemed then to offer an ideal means to commence a new study on the old problems of citronella oil.

3. *The Chemical Composition of Citronella Oils*

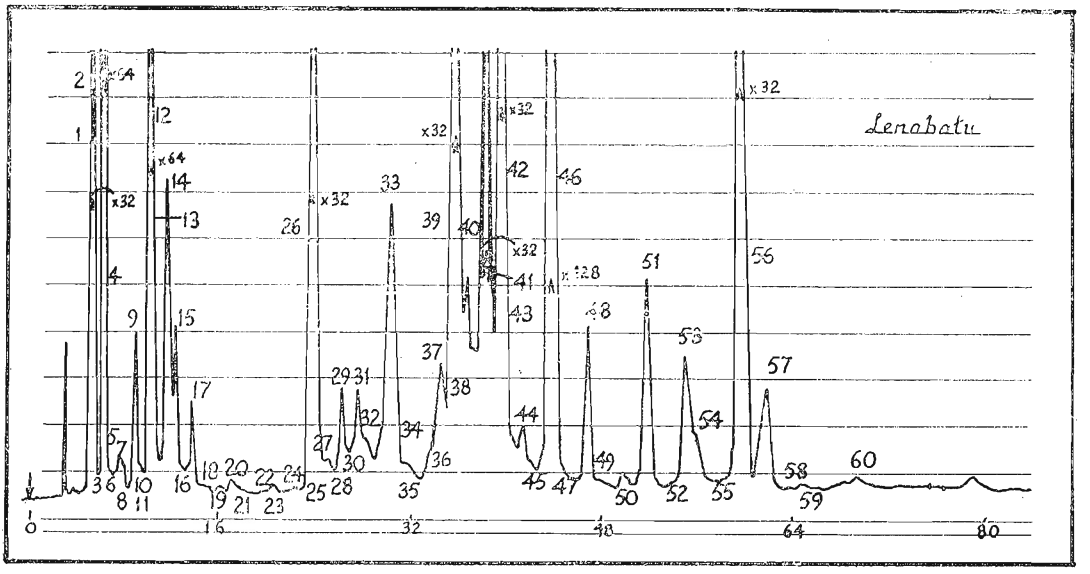
The techniques used in the study of citronella oil[16] were similar to those used previously in a study[2] of the essential oil of cardamom varieties. They were based firstly on obtaining the maximum possible resolution on GC columns (*Figure* 1) ; and secondly, on identifying the various constituents by retention data, and peak enrichment techniques. In peak enrichment, the authentic compounds one at a time were added to the oil prior to injection. The enhance-

ment of the peaks indicated the corresponding positions of the added substances. Such identifications however were necessarily tentative and had to be confirmed by ir spectroscopy. The individual compounds resolved by preparative GLC were collected either into pre-cooled solvents or liquified in capillary tubes cooled to below zero temperatures. Their spectra were then matched with those of authentic substances which too had been purified the same way. In this way the chemical identities of many of the constituents of citronella oil were confirmed. The main differences in chemical composition both qualitative and quantitative, between the oils of Mahapengiri and Lenabatu were also established (*Table* 2). A comparison of the GLC tracings (*Figure* 1) also



Operating parameters for GLC tracings in Figure 1 :

Instrument	:	Varian Aerograph 1740-1 with F.I.D. detector
Column	:	10% Carbowax on Chromosorb W(2.7 × 3,2 mm.)
Programme rate	:	60°—220° at 2°/min., linear
Base attenuation	:	X 16
		(Attenuations above X 16 are indicated).



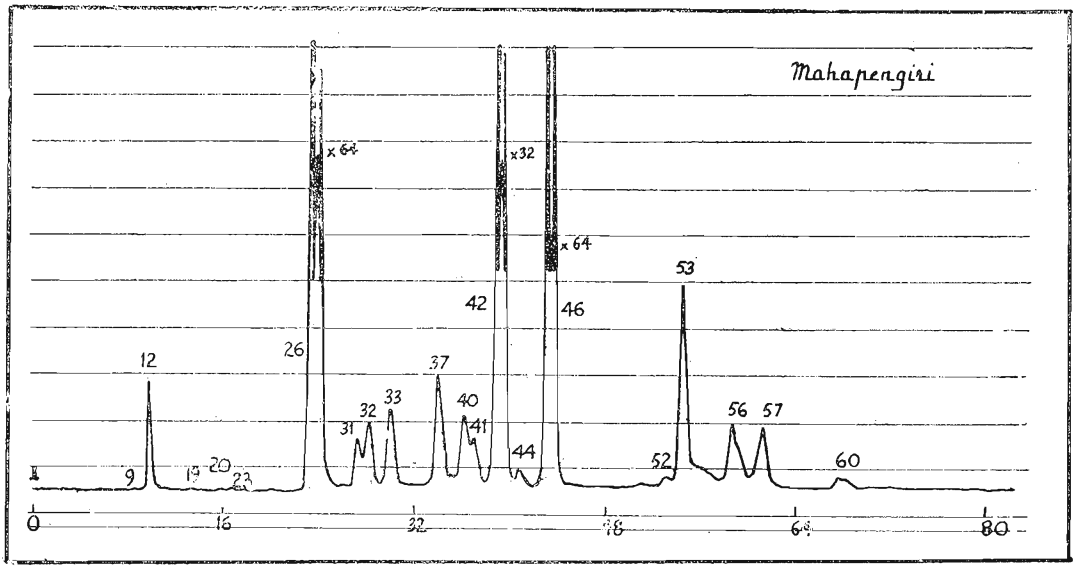


Figure 1. Comparative temperature programmed gas chromatograms of Citronella Oil (Lenabatu type) and Citronella Oil (Mahapengiri) on Carbowax 20 M.
(Operating Condition vide p. 71)

Table 2[16] Chemical Constituents of Citronella oil (Instrumental methods).

Peak No.	Compound	Approximate percentage present in	
		Mahapengiri	Lenabatu
0	Solvent		
1	Tricyclene	—	1.6
2	α – Pinene	—	2.6
4	Camphene	—	8.0
5	β – Pinene	—	trace
6	Sabinene	—	trace
7	Myrcene	—	0.3
8	Car-3-ene	—	trace
9	α – Phellandrene	—	0.8
10	α – Terpinene	—	—
12	Limonene	1.3	9.7
14	*cis*-Ocimene ; γ – Terpinene	—	1.4
15	*trans*-Ocimene ; β – Phellandrene	—	1.8
16	*p* – Cymene	—	trace
17	Terpinolene	—	0.7
20	1—Hexanol	—	0.1
23	Methyl heptenone	trace	0.2
24	Unidentified	—	trace
25	Unidentified	—	trace
26	Citronellal	32.7	5.2
27	Camphor	—	0.5
28	Bourbonene	trace	1.0
29	Linalool	1.5	1.2
30	Linalyl acetate	2.0	0.8
32	α – Terpineol	—	trace
33	β – Caryophyllene	2.1	3.2
34	4 – Terpineol	trace	0.7
35	Menthol	—	trace
36	Unidentified	trace	trace
37	Citronellyl acetate	3.0	1.9
38	Unidentified	—	trace
39	1 – Borneol	trace	6.6
40	Geranyl formate	2.5	4.2
42	Citronellol ; Geranyl acetate*	15.9	8.4
44	Nerol	7.7	0.9
46	Geraniol	23.9	18.0
47	Citronellyl butyrate	trace	trace
48	Geranyl butyrate	—	1.5
50	Nerolidol	—	0.3
51	Methyl eugenol	trace	1.7
53	Elemol	6.0	1.7
56	Methyl iso-eugenol Eugenol	2.3	7.2
57	Unidentified	1.4	1.5
60	Farnesol	0.6	trace

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

reveals these differences. One of the striking differences observed was the presence in the Lenabatu variety of several monoterpene hydrocarbons amounting to more than 20% of the oil as against 3—4%, mainly limonene (V) in the Mahapengiri variety. The presence of a high proportion of hydrocarbons in Lenabatu oil has been recorded previously[12]. Of the monoterpene hydrocarbons in the Lenabatu variety the most abundant was camphene (VI), while the presence of another solid hydrocarbon tricyclene (VII) has been confirmed recently[16].

The other hydrocarbons present were ∝ and β pinenes, sabinene, myrcene, car-3-ene, ∝ and β phellandrene, ∝ and γ—terpinene, *cis* and *trans* ocimene, terpinolene, and *p*-cymene. The frequent co-occurrence of tricyclene and camphene in natural essential oils has been previously recorded by Zavarin and Snajberk[19] in the case of *Abies* cortical oleoresin. The occurrence of these two compounds together with borneol (VIII) and bornyl acetate (IX) in citronella of Lenabatu variety is an indication that the biosynthetic pathway *via* neryl or geranyl pyrophosphate (X) and the 2-bornane carbonium ion (XI) is operative in the case of this plant. (*Chart* 2)

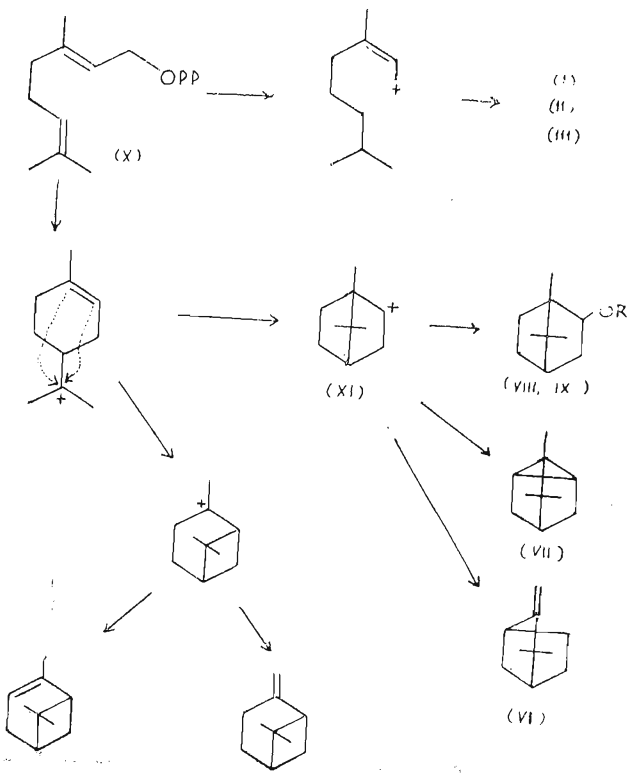
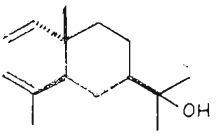


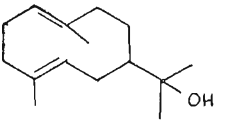
Chart 2. Possible biosynthetic pathways

The Mahapengiri oil contained more oxy-terpenes than the Lenabatu variety. There was no great difference in the amounts of geraniol in the two oils. However the Mahapengiri variety contained much more of citronellal (II) and citronellol (III) which doubtless helped to raise its level of "total acetylisables". Borneol (VIII) was only present as a trace in the Mahapengiri variety but was a significant constituent of Lenabatu-type oil. This solid was no doubt an important contributor to the particular fragrance tones for which the Lenabatu-type is used : *viz* as a soap scenting, and to its reputed "staying" qualities, when thus employed.

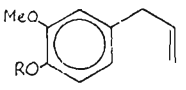
The occurrence of the sesquiterpene elemol (XII) has been recorded before[5] in the case of Mahapengiri oil, and its presence in Lenabatu too has been confirmed[16]. It had been observed by Jones and Sutherland[8] that elemol may not really be the compound that is present in essential oils, but that it may be formed from its thermolabile precursor hedycaryol (XIII).



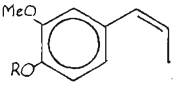
(XII)



(XIII)



(XIV)



(XV)

This is in fact so in the case of citronella oil as, preparative GLC carried out isothermally at 80°C with a low injection temperature of 90° on citronella oil obtained by solvent percolation at ambient temperatures gave hedycaryol and not elemol[16]. Another distinguishing feature of the Lenabatu-type oil is the presence in it of methyl eugenol (XIV, R = Me) and methyl isoeugenol (XV, R = Me). These compounds are the major peaks which appear last on the chromatograms, and are present in only comparatively small quantities in the Mahapengiri variety.

4. *The Story of Adulteration with Kerosene*

For many years it had been assumed that citronella oil produced in Sri Lanka was adulterated with kerosene oil[5]. A publication by Guenther[6] carried this story, illustrated with a variety of anecdotes, from personal experiences of interviews and discussions with exporters in Sri Lanka;

> "There is no secret about the addition of kerosene to Ceylon oils, every exporter and most distillers are perfectly frank about it"

wrote Guenther in 1940 in this report based on a personal survey made in Sri Lanka.

The original method for the detection of kerosene in citronella oil had been devised by the analysts of Schimmel and Co., and introduced in 1898. Two tests known as "old Schimmel's test" and "new Schimmel's test" were originally available. They were both based on solubility in 1—2 volumes of 80% *v/v* ethanol, and the detection of turbidity, in a questionable sample on the further addition of ethanol. The "raised Schimmel's test" was a subsequent development where 5% kerosene was added to the fresh sample and the "old Schimmel's test" applied. Separation of an oily layer was cause for rejection of the sample of oil. The "raised Schimmel's test" is the one mostly employed here by analysts who are called upon to assess the quality of samples of citronella oil. But Guenther[6] recorded that this test, had no practical significance in the trade due to the fact that oils which were slightly old did not readily pass it. Contracts for Sri Lanka citronella oil were mostly based on the "old Schimmel's test[6]. It was felt that the "raised Schimmel's test" was sufficiently exacting to practically exclude any adulterants but there were doubts too as Guenther recorded[6] :

> "Most of the pure freshly distilled oils meet this test without any difficulty *except perhaps certain estate oils which for unknown reasons seem to be less soluble even when freshly distilled**.

Due mainly to the stigma of alleged adulteration the Sri Lanka citronella industry had almost collapsed. Doubts as to the validity of the tests used for the detection of kerosene adulteration were in fact cast by Joachim[7] as early as 1929. He found that oils freshly distilled by him from citronella grass at the Agricultural Experimental Station at Damana in the Batticaloa district and Weligama in the Matara district also failed to pass the various Schimmel's tests. Although the authenticity of these oils was unquestionable, and their content of "total acetylisables expressed as geraniol" were within the average, they were rejected on the market. This caused Joachim to undertake further investigations into the "quality" of Sri Lanka grown citronella, after which he concluded[7] as follows :

* Italics by the present author.

"There is evidently little relationship between the quality of Ceylon citronella oils as gauged by their geraniol contents and their response to Schimmel's test. It has been found that pure unadulterated oils containing at times high geraniol percentages do not pass the test".

Unfortunately Joachim's work had not received adequate attention. The result was that a situation had arisen where perfectly fresh and unadulterated oils were being rejected on the assumption that they were adulterated[6], merely because they failed to pass "tests" which were arbitrary, had no chemical basis, and were at best dubious safety-valves for the buyer.[6] The damage this caused to the citronella industry in Sri Lanka and the island's foreign exchange earnings, cannot be overemphasised. It was in this context that the CISIR's own programme into the chemistry of the essential oils commenced. The first requirement was to study the composition of the oils in detail and thereby develop the necessary knowledge and expertise in analytical techniques, particularly GLC, which would enable the solution of the problems of quality assessment and adulteration. The results briefly discussed earlier[15,16] were achieved as a direct consequence of this approach. The perfection of a method for detection of kerosene then followed[16]. Kerosene itself is a complex mixture of hydrocarbons (*Figure* 2). When a sample of citronella oil containing

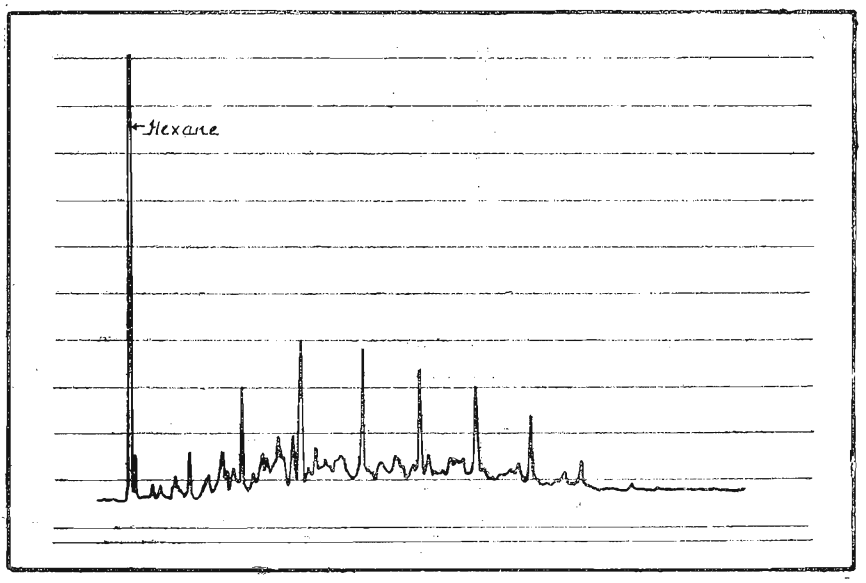


Figure 2. 50% Kerosene in Hexane

Operating Parameters for GLC tracings in Figures 2-4

Instrument	:	Varian Aerograph 1740—1.
Column	:	10% SE.30 on Chromosorb W. (1,5 m × 3,2 mm).
Programme rate	:	60° — 220° at 2°/min. linear.

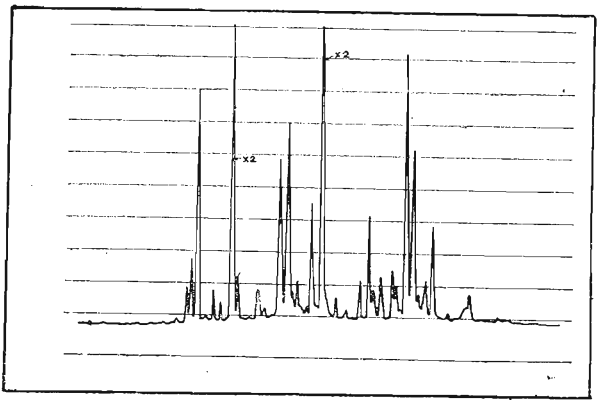


Figure 3 A. Citronella Oil containing 6% Kerosene.

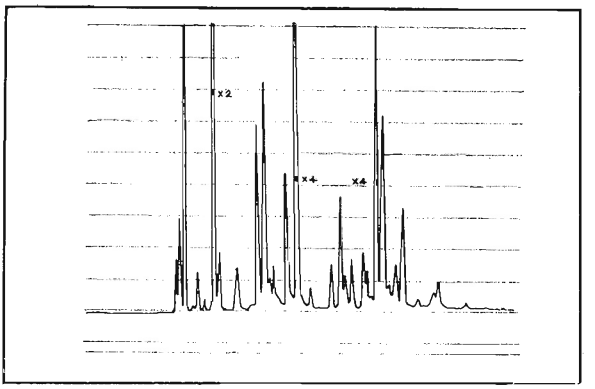


Figure 3 B. GLC profile of pure Citronella Oil (Lenabatu)

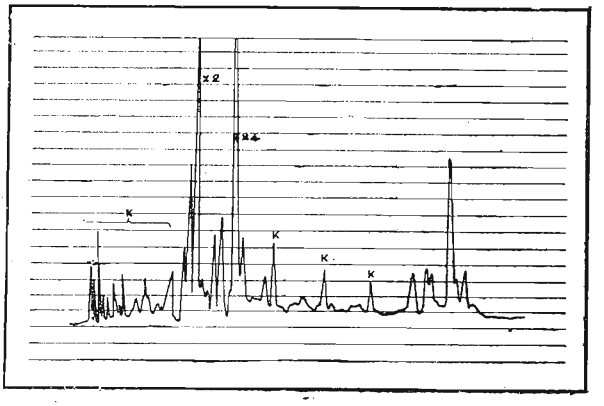


Figure 4. Citronella Oil containing 5% Kerosene, after removal of oxygenated terpenes by preferential adsorption on silica gel. Peaks marked 'K' are due to Kerosene.

kerosene is subjected to GLC the peaks due to the kerosene mostly merge into peaks due to the genuine constituents of citronella. It is thus difficult to detect any adulterant. (*Figure* 3). However when the oxygenated compounds which usually occupy the middle region of the chromatogram are removed by preferential adsorption on silica gel, the hydrocarbon peaks due to kerosene become much more prominent (*Figure* 4). This method is reproducible, and it is possible to detect as little as 2—3 per cent adulteration. It has been used during the past few years and by means of it a great many samples of citronella oil have been tested. The results appear to point to the inescapable conclusion that the practice of adulteration has now been abandoned, in Sri Lanka. No sample containing kerosene has yet been detected. The failure of freshly distilled oils to pass the various solubility tests such as "Schimmel's test" is readily explained by the presence in the Lenabatu-type oil of a very high proportion of natural terpene-hydrocarbons. In respect to solubility in alcohol, these too would react in similar fashion to kerosene hydrocarbons. The GLC method briefly described here can also be extended to determine quantitatively the extent of adulteration with kerosene (if any) up to a lower limit of 2%[18]. However the development of this method served another purpose. This was to exonerate the oil produced in Sri Lanka from alleged adulteration ; for, as Guenther had earlier advised[6] the ability to detect any malpractices at the producers end would benefit the market potential.

5. *Other Benefits of Research*

The systematic study of citronella oil in relation to chemical composition resulted in several other benefits apart from the development of analytical methods for quality assessment. It gave us a basic insight into the chemical composition of this oil, and the extent to which the main constituents varied in proportion. These results were applicable for many purposes. For instance new stills for the distillation of citronella oil were being developed by the CISIR[10,11,17].** The studies enabled the systematic monitoring, using GLC, of the performance of these stills ; the formulation of ideas on the correct methods of preparation of the plant material ; and the optimum time of harvesting of the grass. For instance, it was found that immature grass generally had an even higher content of terpene hydrocarbons than the mature ones ; and that the wilting process was necessary for the production of good quality oil. Seasonal variations are also being observed. In addition the expertise and techniques developed led to the discovery of several possible "varieties" of citronella which consistently gave oils of composition[18] different to either the

** Under a project called IPOMEC (Industrial Products of Minor Export Crops) funded by the Ministries of : Plantation Industries *and* Planning and Employment.

Lenabatu-type or Mahapengiri-type. The morphological differences between these varieties have been recognised, and it would appear that the Lenabatu-type oil is a mixture of the oils from several of these "varieties"†. Experiments on propagating these varieties individually are now in progress,§ and it may be possible to develop varieties containing oil with a high proportion of oxygenated compounds, to suit particular market requirements abroad. The "Mana" or wild citronella varieties are also being included in this study.

6. *Acknowledgement*

The author is grateful to his colleagues, in the Natural Products Section of the Ceylon Institute of Scientific and Industrial Research (CISIR), whose work forms the basis for this paper.

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† These are now termed "chemical races".

§ In collaboration with Dr. W. Herath, Faculty of Agriculture, University of Sri Lanka (Peradeniya Campus), and sponsored by the National Science Council of Sri Lanka.

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