

Essential Oils

II. Infra-Red Spectroscopy in the Analysis of the Volatile Oils of Cinnamon†

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(Paper accepted: 17 April 1974)

Abstract : The chemical composition of cinnamon leaf, stem bark and root bark oils has been examined recently using GLC methods. Infra-red spectroscopy has been used in essential oil studies, mainly as a qualitative technique. In this study, infra-red spectroscopy has been employed to quantitatively estimate the main constituents of the cinnamon oils. These include eugenol, cinnamaldehyde, acetyl eugenol, cinnamyl acetate and benzyl benzoate in leaf oil ; cinnamaldehyde, eugenol and cinnamyl acetate in stem bark oil ; camphor, 1:8 cineole and cinnamaldehyde in root bark oil. The results obtained are in close agreement with those obtained by GLC.

I. Introduction

Infra-red spectroscopy has been widely used in analytical studies on essential oils,²⁻⁵ together with other modern instrumental methods such as GLC, NMR and Mass Spectrometry. Farnov⁶ has discussed the relative merits of the infra-red spectroscopic techniques and chromatographic methods in the analysis of essential oils. He has attempted to identify the spectra of many of the essential oils in terms of the features of the spectra of the major constituents in each of them. For example, the infra-red spectra of lavender oil, lavandin oil and spike oil show striking similarity. The two former oils contain mainly linalool and linalyl acetate and their spectra display the features of the spectra of these individual compounds. Spike oil, on the other hand, contains substantial quantities of 1 : 8 cineole and camphor, in addition to linalool, and its spectrum therefore displays much of their characteristics. Carroll and Price³ have used infra-red spectroscopy as a routine analytical method for analysis of essential oils ; they have used peak intensity measurements in the analysis of citronella oil. Das Gupta and Bhattacharya⁴ have successfully used infra-red spectroscopy as a method of distinguishing between the two geraniols obtained from Java citronella oil and Indian palmarosa oil. Our experience with Sri Lanka cinnamon oils is that the genuineness or otherwise of these oils could be readily revealed by a casual examination of their infra-red spectra.¹⁶

† This Journal, 1 (2) : 67-81 is now considered as Part I of this Series.

* This work will form part of a Ph.D. thesis requirement (University of Sri Lanka, Colombo Campus) of Kanthi H. Fonseka.

Qualitative and semi-quantitative analytical data based on the infra-red spectra of a great many essential oils have been compiled by Bellanato and Hidalgo.² Our studies on the Sri Lanka cinnamon oils have revealed several ambiguities and errors in the assignment of peaks in the spectra of these oils by these workers. Infra-red spectroscopy has hitherto been used successfully to supplement GLC methods in the study of essential oils.¹²⁻¹⁵ However, its role has been primarily qualitative. We have successfully used infra-red spectra as a means of quantitatively determining the major constituents of the cinnamon oils. Our studies are being extended to other essential oils produced in Sri Lanka.

2. Experimental

2.1. Samples of oil

Samples of essential oils analysed were genuine oils obtained from reliable commercial sources in Sri Lanka; some samples were distilled by us from authentic plant material gathered from the cinnamon growing areas in the southern part of the island; these samples were also used in a previous study¹² employing GLC methods.

2.2. Infra-red spectrophotometry

The apparatus used was a Perkin-Elmer model 700 double-beam spectrophotometer with sodium chloride optics. Spectra were recorded as smears or as solutions in chloroform (spectro grade); optimum dilutions and cell thicknesses were pre-selected. An equivalent cell containing chloroform placed in the path of the reference beam was employed to compensate for the absorptions due to the solvent. All solutions were made using Hamilton micro-syringes for measurement of the essential oils and components.

The validity of the Beer-Lambert Law for quantitative analysis was first established by plotting Absorbance at selected frequencies against Concentration, a linear relationship being obtained.¹⁶

2.3. Measurement of absorbance

Absorbance was measured by use of the 'baseline' technique.⁹

2.4. Assignment of absorbance peaks

The assignments of the peaks of the infra-red spectrum of an essential oil as being due to various individual constituents were made by comparison with the characteristic peak frequencies in the individual spectra of the pure constituents. The peak enhancements in the spectra of the essential oil caused by the addition of a small amount of a pure constituent helped to further indicate such peaks as being due to that particular constituent (Figure 1).

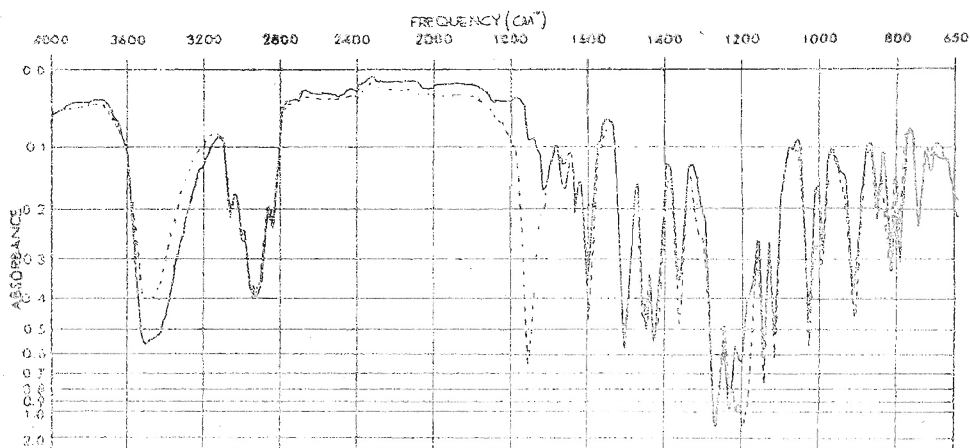


FIGURE 1. Peak enhancement caused by addition of acetyl eugenol to cinnamon leaf oil.
 Continuous line — Cinnamon leaf oil.
 Dotted line — Cinnamon leaf oil to which acetyl eugenol has been added.

2.5. Differential spectroscopy

The peak assignments were confirmed by differential spectroscopy.¹⁰ Here, a small amount of the respective pure compounds was added, one at a time, to the solvent in the reference beam. This resulted in the peaks due to this constituent being reduced (Figure 2). If the quantity added was exactly equal to the amount present in the oil (where the peak is due solely to this compound), the complete disappearance of this peak was observed. Thus, after the estimations by the methods discussed in 2.6. below were carried out, the accuracy of those estimates was checked by obtaining the differential spectrum.

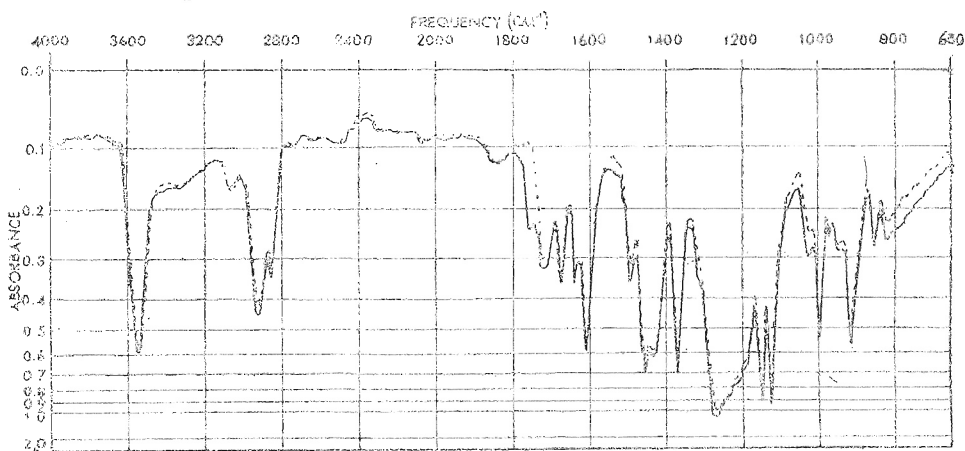


FIGURE 2. Reduction of the intensity of peak 1755 in cinnamon leaf oil caused by the addition of acetyl eugenol to the reference beam.
 Continuous line — Cinnamon leaf oil.
 Dotted line — Cinnamon leaf oil when compensated with acetyl eugenol.

2.6. Quantitation

The quantitations were made graphically by measuring the absorbance and then using this value to read the percentage from a previously plotted calibration curve. But this method was found to be comparatively tedious as a calibration curve was necessary for every compound. Alternatively, the average value of the absorbance at a selected frequency for a pure component was used to measure the amount of the same substance present in the essential oil by making use of the Beer-Lambert Law.

By this law,

$$I = I_0 e^{-kcl}$$

$$\frac{I_0}{I} = e^{kcl}$$

$$\log_e \frac{I_0}{I} = kcl$$

$$\text{i.e. } A = kcl \quad (1)$$

where I_0 = Intensity of incident light

I = Intensity of transmitted light

k = Extinction coefficient

c = Concentration in g/l

l = Path length in cm

A = Absorbance or optical density

For a pure substance if the absorbance A is determined for a known c and known l , then equation (1) could be used to find the unknown concentration c' in the essential oil if the absorbance A' of the essential oil is determined for a known or same l .

TABLE I. Observed absorbance A for path length 0.5 cm (l).

Compound	% Concentration	Absorbance A	Frequency cm^{-1} selected
Eugenol	3.33	0.626	3540
Acetyl eugenol	0.41	0.589	1750
Cinnamyl acetate	0.41	0.840	1730
Benzyl benzoate	0.41	0.765	1715
Cinnamaldehyde	0.41	0.729	1670
Camphor	0.83	0.720	1730
1 : 8 cineole	3.33	0.652	975

2.7. Quantitation technique with changing baseline

In some cases, although there were sharp peaks due to certain single components in the spectra of the essential oil, the direct estimation of that component by making use of equation (1) was not possible. This was due to the baseline of the peak in the spectrum of the essential oil changing markedly from that of the pure component. In such cases, it was possible to obtain an estimate by adding a small quantity (measured amount) of the pure component to the essential oil and measuring the new absorbance. It was found that the addition of a little of the pure component to the essential oil did not in most cases change the baseline of the peak given by the essential oil itself to any appreciable extent. As an example, say the required concentration of a pure substance in the oil is X g/l. Then the absorbance is given by:

$$A = k c l = K X \quad (2)$$

In this equation, A can be found experimentally. If a little amount X_0 is added to the oil, with the base line remaining virtually the same, the new absorbance

$$A' = K (X + X_0) \quad (3)$$

Hence, by measuring A' , and knowing X_0 , X can be found from the two equations (2) and (3).

Our estimation of eugenol in the cinnamon root bark oil was based on this method where we employed the absorbance at 1505 cm^{-1} .

2.8. Quantitation with multiple absorbance

When a certain peak is due to two or more constituents and the constituents are correctly assigned, the additive relationship of absorbance could be used for estimation.¹¹

$$A_\lambda = \sum_i k_{i\lambda} c_i l \quad (4)$$

or for two components

$$A_\lambda = k_{1\lambda} c_1 l + k_{2\lambda} c_2 l \quad (5)$$

where both components absorb at the same frequency. In our estimation of 1 : 8 cineole in cinnamon root bark oil, equation (5) was employed. Here, the peak at 975 cm^{-1} is contributed by cinnamaldehyde and 1 : 8 cineole. Since the concentration of cinnamaldehyde can be found from other peaks, this 975 cm^{-1} absorbance gave an easy estimation of 1 : 8 cineole. From equation (5), $A = K_1 c_1 + K_2 c_2$. If the subscripts 1 and 2 refer to cinnamaldehyde and 1 : 8 cineole respectively, c_1 can be found by measuring A for the oil and K_1 and K_2 for the pure components.

TABLE 2. Peak assignments for qualitative analysis of cinnamon leaf oil.

Frequency cm^{-1}	Contributing components
3500 } 3400 } 3065 } 3000 } 2970 } 2940 } 2910 } 2840 } 2740 }	Eugenol (O-H stretch) Eugenol (=C-H stretch aromatic) Eugenol (alkenes) Eugenol (aliphatic CH vibrations) Eugenol Eugenol (mainly) + Acetyl eugenol, Safrol, Linalool, etc. Eugenol (mainly) + Acetyl eugenol, Cinnamaldehyde, etc. Cinnamaldehyde
	$\begin{array}{c} \text{H} \\ \\ (-\text{C} \text{ CH stretch}) + \text{Eugenol} \\ \\ \text{O} \end{array}$
2640	Eugenol
2580	Eugenol
2540	Eugenol
2400	Eugenol
2300	Eugenol
2250	Eugenol
2060	Eugenol
2160	Eugenol
1970	Eugenol
1840	Eugenol
1755	Acetyl eugenol (C=O stretch)
1735	Cinnamyl acetate (C=O stretch)
1715	Benzyl benzoate (C=O stretch)
1675	Cinnamaldehyde + Eugenol (C=O stretch in Cinnamaldehyde)
1635	Eugenol
1600	Eugenol
1510	Eugenol
1490	Eugenol
1460	Eugenol
1450	Eugenol
1430	Eugenol
1365	Eugenol
1270	Eugenol + Benzyl benzoate (C—O—stretch; aryl in both)
1230	(Eugenol + Cinnamyl acetate (C—O—stretch in both))
1210	Eugenol + Acetyl eugenol (C—O—stretch)
1200	Eugenol + Acetyl eugenol (C—O—stretch)
1180 (Sh)	Eugenol
1150	Eugenol
1120	Eugenol
1070	Eugenol
1030	Eugenol (—O CH ₃)
990	Eugenol
975	Cinnamaldehyde + Cinnamyl acetate (trans CH deformation)
950	Eugenol (—CH=CH—CH ₃)
915	Eugenol + Safrol
850	Eugenol
815	Eugenol
795	Eugenol
745	Eugenol
710	Eugenol
690	Cinnamaldehyde + Cinnamyl acetate

TABLE 3. Peak assignments for qualitative analysis of cinnamon stem bark oil.

Frequency cm ⁻¹	Contributing components
3500 } 3400 }	Eugenol + Linalool, Cinnamyl alcohol—OH stretch }
3330	Cinnamaldehyde
3070	Cinnamaldehyde
3040	Cinnamaldehyde + Cinnamyl acetate
2970	Eugenol (C-H vibrations)
2940	Cinnamyl acetate + Eugenol
2860	Cinnamyl acetate
2825	Cinnamaldehyde ⁸ (overtone of the aldehydic bending vibration)
2740	Cinnamaldehyde (Aldehydic C-H stretch)
2450	Cinnamaldehyde
2250	Cinnamaldehyde
1970	Cinnamaldehyde
1950	Cinnamaldehyde
1880	Cinnamaldehyde
1800	Cinnamaldehyde
1730	Cinnamyl acetate (C=O stretch α β unsaturated)
1675	Cinnamaldehyde
1620	Cinnamaldehyde
1600	Cinnamaldehyde
1570	Cinnamaldehyde
1510	Cinnamaldehyde
1490	Cinnamaldehyde + Cinnamyl acetate
1460	Eugenol
1450	Cinnamaldehyde + Cinnamyl acetate
1385	Cinnamyl acetate
1365	Cinnamyl acetate
1330	Cinnamaldehyde
1300	Cinnamaldehyde
1270	Eugenol
1250	Cinnamaldehyde
1240	Cinnamyl acetate + Eugenol
1210	Cinnamyl acetate + Cinnamaldehyde + Eugenol
1180	Cinnamaldehyde
1160	Cinnamaldehyde
1150	Cinnamaldehyde + Eugenol
1120	Cinnamaldehyde
1070	Cinnamaldehyde
1030	Cinnamyl acetate + Eugenol
1010	Cinnamaldehyde
970	Cinnamaldehyde + Cinnamyl acetate (trans CH deformation)
910	Cinnamaldehyde + Eugenol
850	Cinnamaldehyde + Eugenol
820	Cinnamyl acetate
745	Cinnamaldehyde + Cinnamyl acetate
690	Cinnamaldehyde + Cinnamyl acetate

TABLE 4. Peak assignments for cinnamon root bark oil.

Frequency cm ⁻¹	Contributing compounds
3540 } 3400 }	Eugenol Terpineol
3070	Cinnamaldehyde, Eugenol
2960	Camphor, 1 : 8 Cineole, Eugenol
2900	Camphor, 1 : 8 Cineole, Terpineol, Eugenol
2875	Camphor
2740	Cinnamaldehyde
1740	Camphor
1675	Cinnamaldehyde
1620	Cinnamaldehyde
1600	Cinnamaldehyde, Eugenol
1505	Eugenol
1450	Camphor, 1 : 8 Cineole, Terpineol, Cinnamaldehyde
1415	Camphor
1390	Camphor, 1 : 8 Cineole
1370	Camphor, 1 : 8 Cineole, Terpineol, Eugenol
1320	Camphor
1300	Camphor 1 : 8 Cineole, Cinnamaldehyde, Terpineol
1270	Camphor, 1 : 8 Cineole, Eugenol
1240	1 : 8 Cineole, Eugenol
1210	Eugenol
1200	Eugenol
1165	1 : 8 Cineole, Terpineol
1150	Eugenol, Terpineol
1135	Cinnamaldehyde, Eugenol, Terpineol
1120	Eugenol, Terpineol, Cinnamaldehyde
1095	Camphor
1080	1 : 8 Cineole, Cinnamaldehyde
1045	Camphor, 1 : 8 Cineole
1020	Camphor, 1 : 8 Cineole, Eugenol
980	1 : 8 Cineole, Cinnamaldehyde
950	Camphor, Cinnamaldehyde, Terpineol
935	Terpineol
910	Eugenol, Terpineol
890	1 : 8 Cineole
845	1 : 8 Cineole, Eugenol
815	Terpineol
795	Eugenol
745	Camphor, Cinnamaldehyde, Eugenol
690	Cinnamaldehyde

TABLE 5. Comparison of results by IR and GLC methods.
(I) Cinnamon leaf oil

Compound	Percentage	
	by IR	by GLC
Eugenol	79.9	80.0
Acetyl eugenol	3.7	2.1
Cinnamyl acetate	3.2	1.8
Benzyl benzoate	4.5	3.4
Cinnamaldehyde	2.0	2.4

(II) Cinnamon bark oil

Compound	Percentage	
	by IR	by GLC
Cinnamaldehyde	63.5	63.0
Cinnamyl acetate	13.0	5.0
Eugenol	8.0	10.0

(III) Cinnamon root bark oil

Compound	Percentage	
	by IR	by GLC
Camphor	52.5	59.9
1 : 8 Cineole	12.0	19.2
Eugenol	5.0	5.0
Cinnamaldehyde	3.3	3.9

3. Results and Discussion

3.1. Cinnamon leaf oil

The infra-red spectrum of a sample of Sri Lanka cinnamon leaf oil is strikingly similar to the spectrum of eugenol, its main constituent (Figures 3 and 4). However, the appearance of four distinct peaks in the carbonyl region ($1750-1670\text{ cm}^{-1}$) readily distinguishes cinnamon leaf oil from eugenol. The OH stretching frequency at 3540 cm^{-1} is suitable for quantitative assay of eugenol in cinnamon leaf oil, as contributions from other hydroxylic compounds are negligible. The peak due to OH (stretch) appears as a broad band in the spectrum from a film of the oil, but in spectra from dilute solutions of the oil in many organic solvents, it becomes narrow and sharp. In 3.33% chloroform solutions employed in this study, it gave a reproducible absorbance of 0.626 for a path length of 0.5 mm. Accordingly, this peak was used to estimate the eugenol content of cinnamon leaf oil, using the Beer-Lambert relationship. After each estimation, the differential spectrum of the leaf oil was recorded with the estimated amount of eugenol added to the solvent in the reference beam.

The complete disappearance of all peaks assignable to eugenol confirmed the accuracy of the estimation. When the estimate was incorrect, i.e. when it was too high, or too low, this was indicated by the appearance of negative, i.e. or residual peaks respectively. Beside the OH stretch peak at 3540 cm^{-1} , other sharp peaks due to eugenol could also be employed for its quantitation. Beside eugenol, all other peak assignments too were confirmed in similar fashion by differential spectra. By this means, several assignments reported by Bellanato and Hidalgo² were found to be erroneous. These authors have assigned peaks at 2910 and 2840 cm^{-1} as due to eugenol, but in fact other compounds are also partly responsible for these peaks (Table 2). Likewise, the peak at 2740 cm^{-1} again assigned only to eugenol has a contribution from cinnamaldehyde as well (aldehyde C-H stretch).

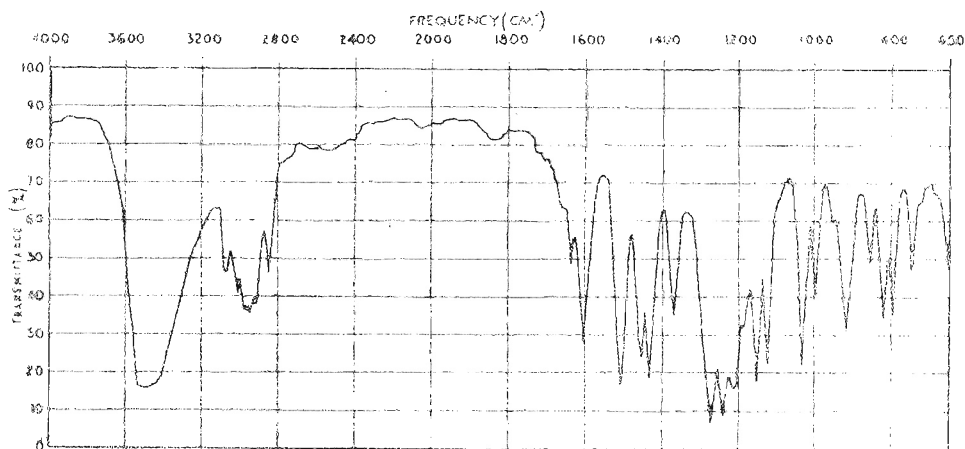


FIGURE 3. Spectrum of eugenol.

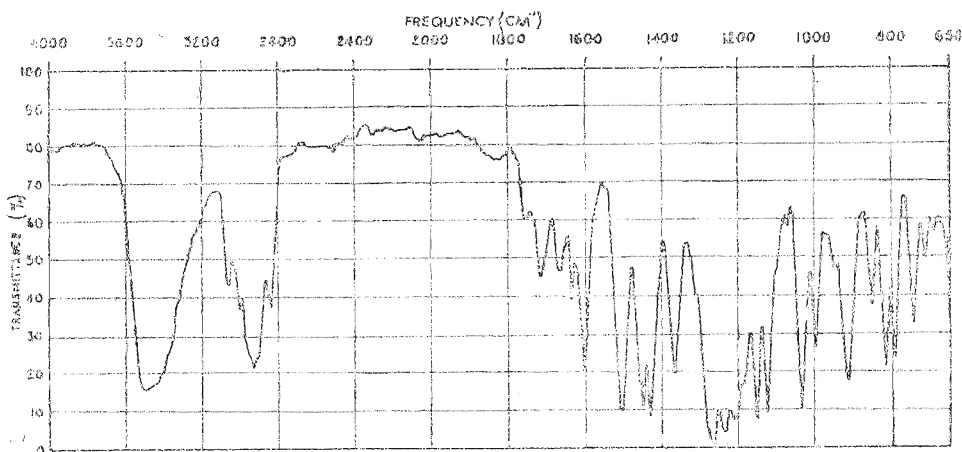


FIGURE 4. Spectrum of cinnamon leaf oil.

In the carbonyl region, there are four peaks which appear at 1755, 1735 (shoulder) 1715 and 1675 cm^{-1} . Using the methods described above, these have been confirmed as substantially due to acetyl eugenol, cinnamyl acetate, benzyl benzoate and cinnamaldehyde respectively. The presence of acetyl eugenol and benzyl benzoate as a constituent of cinnamon leaf oil has not been recorded by Bellanato and Hidalgo² in their IR studies. Although Angmor *et al*² report the presence of acetyl eugenol in their GLC studies on cinnamon leaf oil, they do not record the presence of benzyl benzoate. However, it has been reported in the early work of Guenther⁷ and Glichitch⁶ and confirmed recently by GLC studies as well.¹² The 1675 cm^{-1} peak is not solely due to cinnamaldehyde, there being a very small contribution to it from eugenol. Previous assignment² of the peak 1630 cm^{-1} as due to cinnamyl acetate is incorrect, as there is no peak at 1630 in the IR spectrum of pure cinnamyl acetate. The peak 1510 cm^{-1} previously assigned to safrole is due to both eugenol and safrole. It has been noticed that intense peaks in the spectra of benzyl benzoate, cinnamyl acetate and acetyl eugenol are at 1270, 1230 and 1210 cm^{-1} respectively. Hence in the spectrum of cinnamon leaf oil in which these compounds are present, there should be certain contributions in the carbonyl region due to each of them. This was verified by means of differential spectra. Further, the 975 cm^{-1} peak has been found to be due to cinnamaldehyde as well as to cinnamyl acetate and cinnamyl alcohol. This intense peak is due to *trans* CH deformation.

3.2. Cinnamon stem bark oil

Of the three essential oils obtainable from cinnamon, cinnamon stem bark oil is the most expensive. This is due to its pleasant and acceptable aroma and its use in the food and beverage industries. The major constituent of cinnamon bark oil is cinnamaldehyde and in the infra-red spectrum of cinnamon stem bark oil, the dominant peaks are due to it (Figures 5 to 8). Some peaks due to cinnamyl acetate, eugenol and linalool are also displayed depending on the quality of the oil. The content of cinnamaldehyde and even the authenticity of cinnamon stem bark oil could be readily evaluated from an infra-red spectrum of it in the form of a film. The infra-red spectra of good quality cinnamon stem bark oil bears a striking resemblance to that of cinnamaldehyde (Figures 5 and 6). Here, the intensities of the peaks 2740 cm^{-1} (medium) 1675 cm^{-1} (strong) and 975 cm^{-1} (strong) agree very closely to those of cinnamaldehyde. In certain samples of the oil the terpenic fraction was found to be high thereby reducing its quality. This may be due to faulty distillation or the use of immature bark for distillation. In this case, the intensities of C-H stretching absorptions which appear in the range 2970-2945 cm^{-1} are very much higher (Figure 7). Also, the peaks 2740 and 1670 cm^{-1} become reduced in intensity. These peaks are due to aldehydic C-H stretch and carbonyl stretch from cinnamaldehyde. Sometimes the bark is distilled along with twigs, in which case the quality of the oil becomes poorer and the oil thus obtained is known in Sri Lanka as "katta thel" (Figure 8). This oil contains a higher amount of eugenol and is readily detectable as there are

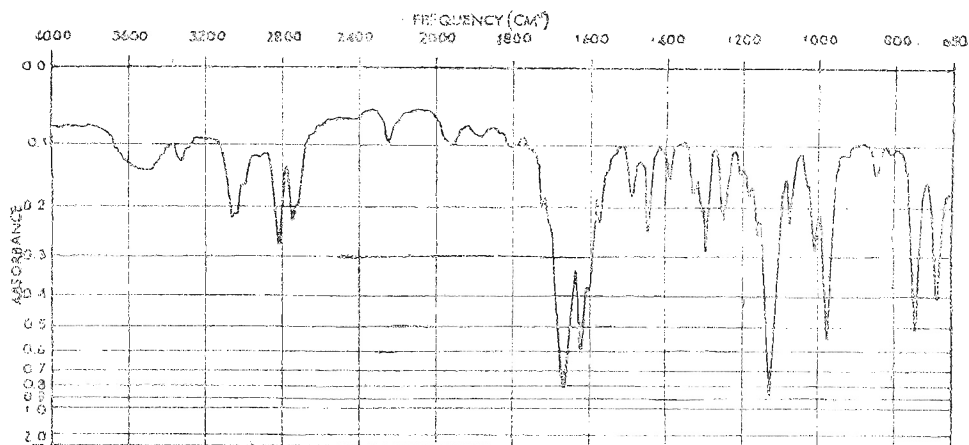


FIGURE 5. Spectrum of cinnamaldehyde.

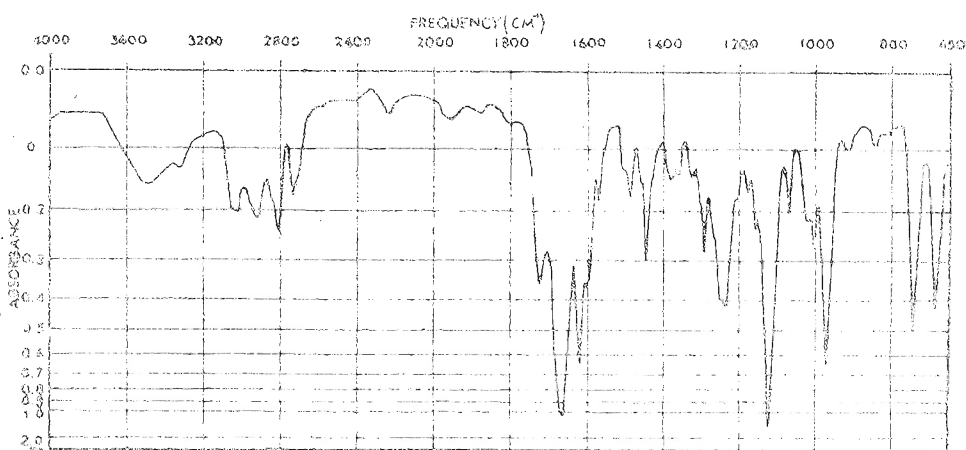


FIGURE 6. Spectrum of cinnamon stem bark oil.

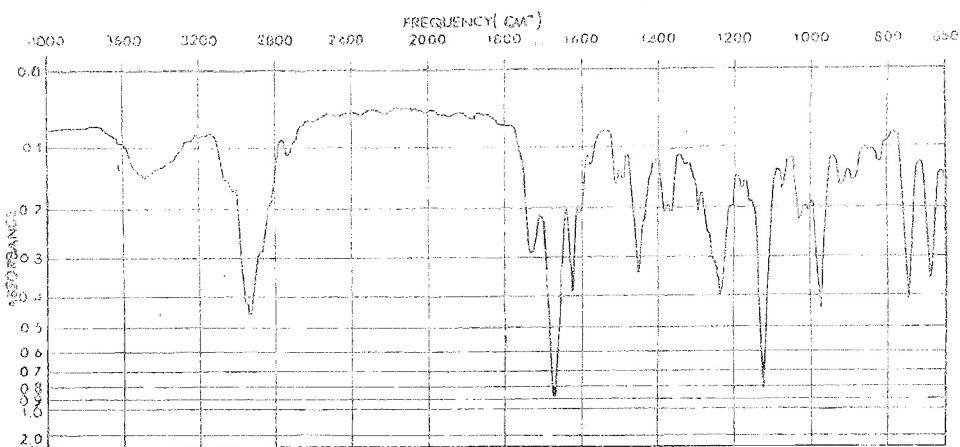


FIGURE 7. Cinnamon bark oil containing a high proportion of terpenes.

enhanced eugenol peaks 3540, 1500, 1420, 1270, 1230 and 1030 cm^{-1} . A similar situation arises when there are adulterations of cinnamon stem bark oil with the cheaper leaf oil. In this case too eugenol peaks become prominent. The broad band 3540-3400 cm^{-1} is contributed by eugenol and other hydroxylic compounds present such as linalool and cinnamyl alcohol. However, it has been assigned previously² as due to eugenol entirely. Similarly, previous assignment of peaks at 3040, 2940, 2860, 1620, 1510, 1490, 1450, 1385, 1250, 1240, 1210, 1180, 1150, 820 and 690 cm^{-1} were found to be erroneous, and the correct assignments are given in Table 3. Our assignments are based on the methods discussed previously (2.4). The quantitation of cinnamaldehyde was carried out by using the peak at 2740 cm^{-1} . This could also be achieved from a graph of absorbance versus percentage of cinnamaldehyde. The carbonyl absorbance at 1675 cm^{-1} could as well be employed for this purpose.

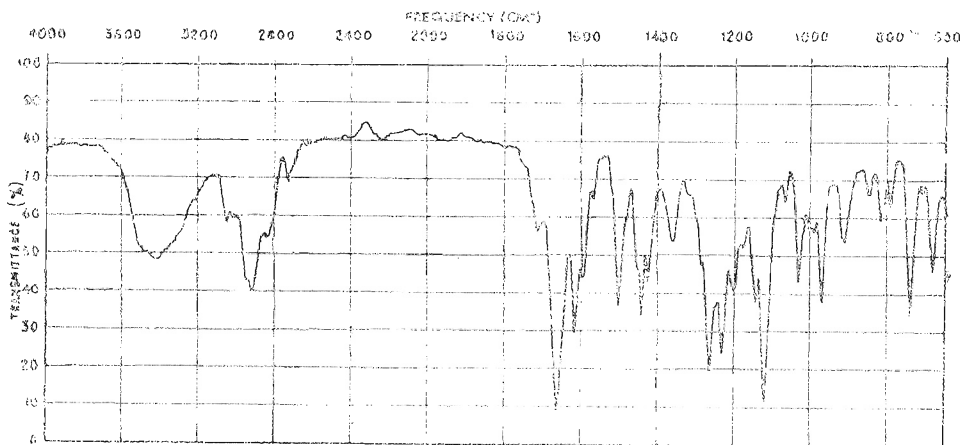


FIGURE 8. Cinnamon bark oil of poor quality ("Katta Thel").

3.3. Cinnamon root bark oil

The infra-red spectrum of cinnamon root bark oil appears very similar to that of camphor, the principal constituent in it (Figures 9 and 10); there are additional peaks due to 1 : 8 cineole and others. The estimation of camphor in root bark oil was accomplished by using the carbonyl absorbance at 1740 cm^{-1} , and cinnamaldehyde and 1 : 8 cineole by use of the absorbance at 1575 cm^{-1} , and 980 cm^{-1} respectively. Since the peak at 980 cm^{-1} is contributed by both cinnamaldehyde and 1 : 8 cineole, the estimation of 1 : 8 cineole was achieved by using the method described earlier (2.4). The eugenol content was estimated by using the method discussed in 2.8.

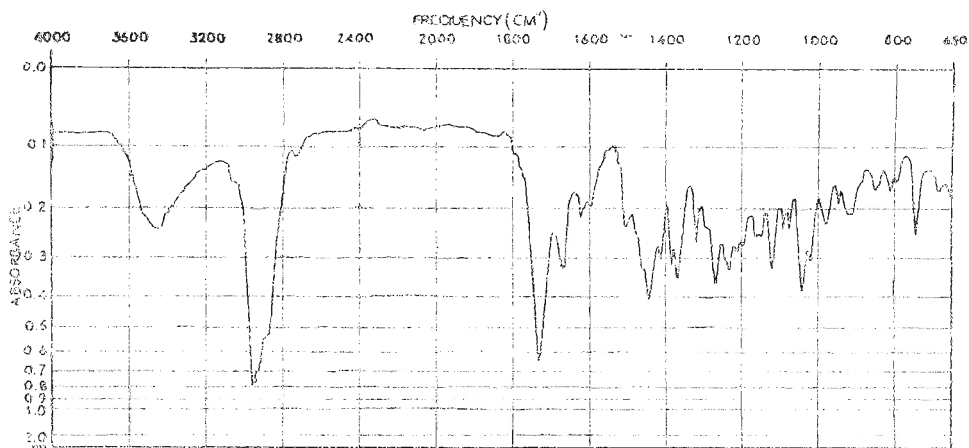


FIGURE 9. Spectrum of root bark oil.

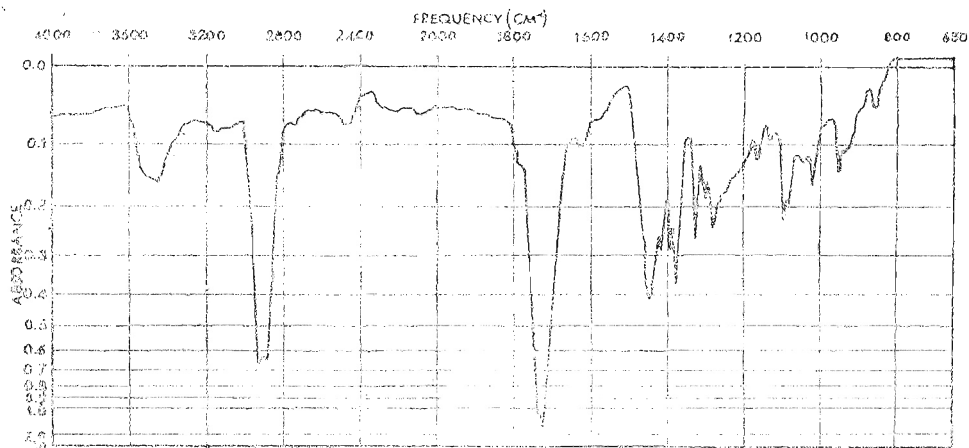


FIGURE 10. Spectrum of camphor.

4. Conclusion

IR spectroscopy can be employed to give a rapid and accurate estimate of the principal constituents of the essential oils of cinnamon. The method affords another valuable parameter in the assessment of quality in essential oils, and is particularly suitable for routine application.

Acknowledgements

This work has been partly supported by grants from the Ministry of Plantation Industries and the National Science Council of Sri Lanka. The authors are grateful to Miss Clodagh Nethsingha, Chief Librarian, C.I.S.I.R. for assistance with the literature, to Mrs. Shirani Colombage and Mr. P.C.M. Fernando for help in the preparation of the manuscript and to Mr. I. N. Fernando for valuable technical assistance in regard to the infra-red spectroscopic methods.

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