

Determination of *o*-methoxycinnamaldehyde in cassia oil by infra-red spectrophotometry

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The determination of *o*-methoxycinnamaldehyde is described, based upon an absorption measurement at 1266 cm^{-1} in the infra-red region of the spectrum together with the normal assay for total aldehydes. Comparison of the absorption at 1266 and 1312 cm^{-1} may be used qualitatively to distinguish between oils derived from the bark of *Cinnamomum cassia* Blume and those from the leaves and twigs.

OIL of cassia is the volatile oil distilled from the leaves and twigs of *Cinnamomum cassia* Blume, and it consists mainly of cinnamaldehyde together with *o*-methoxycinnamaldehyde, cinnamic acid and esters (Guenther, 1950). The composition of the bark oil is similar but Darlington & Christensen (1944) have noted that the percentage of *o*-methoxycinnamaldehyde is much less than that of the leaf and twig oil. The determination was made using a modification of Zeisel's method for the methoxyl group and special precautions were taken by the authors to ensure reliable results.

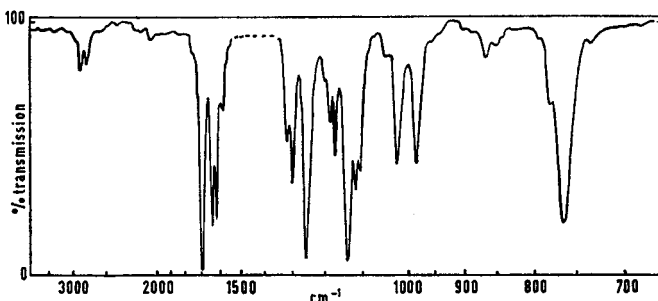


FIG. 1. 5 % w/v *o*-methoxycinnamaldehyde in acetonitrile (0.1 mm cell)

Infra-red spectrophotometry has not been used for the determination of *o*-methoxycinnamaldehyde and this investigation followed the preparation of a small sample of the aldehyde for photochemical study. Comparison of the infra-red absorption curve (Fig. 1) with that of cinnamaldehyde (Fig. 2) showed that only one absorption band, viz. that at about 1266 cm^{-1} , was likely to be of value. Cinnamaldehyde showed absorption in this region but the intensity was very much less than that due to *o*-methoxycinnamaldehyde. The difference was deemed sufficient to justify the use of a correction for the cinnamaldehyde.

Two determinations are therefore necessary; infra-red absorption for total aldehyde calculated as *o*-methoxycinnamaldehyde and a chemical (or physical) method for total aldehyde calculated as cinnamaldehyde. The latter assay is normally carried out in assessing the quality of an oil

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and the final expression for *o*-methoxycinnamaldehyde will vary slightly according to the method adopted. The formula for calculation of the true percentage of *o*-methoxycinnamaldehyde is readily derived, bearing in mind that with hydroxylamine hydrochloride for example 162.2 g *o*-methoxycinnamaldehyde = 132.2 g cinnamaldehyde and from infra-red analysis 100% cinnamaldehyde = 6.8% *o*-methoxycinnamaldehyde. Using ultra-violet absorption at the wavelength (286 m μ) for *cinnamaldehyde*, *E* (1%, 1 cm) for *o*-methoxycinnamaldehyde was found to be 1110.

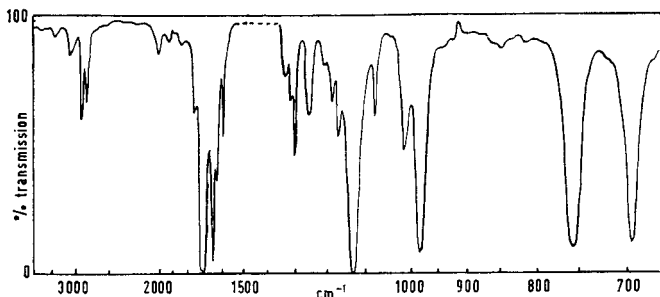


FIG. 2. 10.4% w/v cinnamaldehyde in acetonitrile (0.1 mm cell)

Preliminary experiments indicated that the presence of terpenes and esters in the form of an aldehyde-free oil (see below) caused a decrease in the extinctions of solutions of *o*-methoxycinnamaldehyde and of cinnamaldehyde although the relationship between them remained the same. It is desirable therefore to prepare the calibration curve with mixtures closely akin to natural oils. In the absence of a suitable quantity of cassia oil, the non-aldehyde fraction of cinnamon oil was isolated for this purpose; it contained a small quantity of esters (absorption at 1740 cm⁻¹) which were probably formed during its isolation. It was used to prepare the standard oil for admixture with varying quantities of *o*-methoxycinnamaldehyde. Beer's law applied over the concentration range used but the curve is not reproduced here as it may be obtained from Table 1 (columns 6 and 7).

Experimental

Infra-red absorption spectra were measured by means of a Perkin-Elmer Infracord, model 137, fitted with sodium chloride optics; the accurate figures 1312 and 1266 cm⁻¹ were obtained on a Perkin-Elmer model 237 instrument; ultra-violet absorption spectra were recorded on an Optica CF4D.R. instrument. Melting-points are uncorrected.

o-Methoxycinnamaldehyde. The aldehyde was prepared in low yield by the method of Vorländer & Gieseler (1929) for *p*-methoxycinnamaldehyde as pale yellow glistening flakes, m.p. 44–46°; (phenylhydrazone, m.p. 116–118°) (Bertram & Kürsten, 1895, give m.p. 45–46° and 116–117° respectively); (OMe) 19.0%, Calc. for C₉H₇O (OMe), 19.1%; *E* (1%, 1 cm) at 281 m μ , 1160 (max.); *E* (1%, 1 cm) at 286 m μ = 1110.

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Aldehyde-free oil. Cinnamon oil (50 ml) in methanol (500 ml) was treated with Girard's reagent T (50 g) and boiled under reflux for 1 hr. The mixture was cooled, water (1000 ml) was added and the oil was extracted with light petroleum (b.p. 40–60°). The ether extract was washed once with dilute solution of sodium hydroxide (1%, 50 ml) and with brine (3 × 50 ml). The ether extract was dried (Na₂SO₄) and evaporated to yield a brown aldehyde-free oil (9 g, weak ester absorption at 1740 cm⁻¹).

TABLE 1. DATA USED IN CONSTRUCTION OF CALIBRATION CURVE

M* (mg)	Standard oil (mg)	Final volume (ml)	Cinnamaldehyde (mg/ml)	<i>E</i>	<i>E</i> for M* only	M* mg/ml of final solution
0	517.9	5.00	88.3	0.127	—	—
17.6	482.3	5.00	82.3	0.192	0.074	3.52
17.3	185.2	2.00	77.9	0.301	0.189	8.65
24.8	172.3	2.00	74.5	0.370	0.263	12.4
84.9	414.1	5.00	70.7	0.450	0.348	17.0
37.8	170.6	2.00	69.7	0.507	0.407	18.9

* M = *o*-Methoxycinnamaldehyde.

Standard oil free from o-methoxycinnamaldehyde. The aldehyde-free oil was mixed with redistilled cinnamaldehyde so that the mixture contained 85.2% w/w cinnamaldehyde.

Calibration curve. Accurately weighed quantities of *o*-methoxycinnamaldehyde were added to weighed amounts of standard oil and dissolved in acetonitrile at a standard temperature. Table 1 incorporates the quantities and volumes. The absorption curve of each solution was recorded over the region 2000 to 1000 cm⁻¹ using a 0.1 mm cell. A reference cell with acetonitrile was used to compensate for solvent absorption.

The extinction of each solution was calculated for the peak absorption at about 1260 cm⁻¹ using the base line technique (cf. Cross, Gunn & Stevens, 1957). The calculated extinction for the cinnamaldehyde was subtracted from the observed extinction and the difference was plotted against the concentration of *o*-methoxycinnamaldehyde expressed in mg/ml of final solution. All the results are recorded in Table 1.

Correction for cinnamaldehyde. The extinctions of solutions of cinnamaldehyde in acetonitrile were compared with those for *o*-methoxycinnamaldehyde. 100% cinnamaldehyde was equivalent to 6.8% *o*-methoxycinnamaldehyde.

Method. Prepare, at a standard temperature, 2.00 ml of a 10% w/v solution of the oil in acetonitrile and record the absorption spectrum over the region 2000–1000 cm⁻¹. Calculate the extinction using the base line technique and read off from the calibration curve the apparent content of *o*-methoxycinnamaldehyde in mg/ml of final solution. Calculate the apparent percentage of the aldehyde (B).

Determine the percentage of total aldehyde, calculated as cinnamaldehyde (A) by a suitable method. The true percentage of *o*-methoxycinnamaldehyde is given by the expression % *o*-methoxycinnamaldehyde

= 1.06 B—0.072 A when hydroxylamine hydrochloride is used for the determination of A. When ultra-violet spectrophotometry is used the percentage is given by 1.04 B—0.071 A.

The results obtained on various samples are recorded in Table 2, which also includes the results of chemical determinations of the methoxyl group by a modified Zeisel method.

TABLE 2. RESULTS

Sample	% <i>o</i> -Methoxycinnamaldehyde		% Total aldehyde calculated as cinnamaldehyde	
	By infra-red	By methoxyl determination	By hydroxylamine	By ultra-violet spectrophotometry
Bark oil A	3.7; 3.7; 3.2	3.60; 3.74; 3.80	86.5	84.5
B	3.2	3.20; 3.27; 3.12	85.7	85.0
C	1.5; 1.45	1.59; 1.49; 1.50	81.5	79.1
D	3.7; 3.7	3.40; 3.0; 3.40	—	82.8
Leaf and twig oil ..	11.6; 11.4	11.24; 11.16; 11.20	85.7	—
Mixture I	10.0; 10.3	10.0 } Theory	—	—
Mixture II	4.8; 4.8	5.0 } Theory	—	—
Cinnamon oil ..	not detected	—	61.1	—

Results and discussion

The results for cassia oils (Table 2) fall into the two categories observed by Darlington & Christensen (1944) *viz.* a low percentage of *o*-methoxycinnamaldehyde in the bark oils and a much higher percentage in the leaf and twig oil. It would appear that genuine cassia oil contains high percentages of *o*-methoxycinnamaldehyde as observed by Dodge (1939). It is unfortunate that we were able to obtain only one sample of the leaf and twig oil so that some caution must be observed in drawing comparisons between the two oils. The value of infra-red absorption in this respect lies in the fact that a simple *qualitative* run on a liquid film may distinguish the bark from the leaf and twig oil because the band at about 1312 cm^{-1} acts as an internal standard. In bark oils the band is more intense than that at about 1260 cm^{-1} , whereas in the leaf and twig oil the converse is true.

It was hoped that examination of the infra-red absorption curves over the region 800 to 700 cm^{-1} might also provide information on the cinnamaldehyde content, hence the interest in selecting a solvent which was sufficiently transparent in that region. On re-appraisal of the many possible variables, *viz.* the number and proportion of mono-substituted benzene compounds other than cinnamaldehyde in cassia oil (Guenther, 1950), it was decided to abandon this approach, attractive though it seemed. Total aldehyde was therefore determined by hydroxylamine hydrochloride and by direct ultra-violet spectrophotometry. These results are included in Table 2.

The methoxyl content of the oils was checked by a modified Zeisel method and results in agreement with those found by infra-red spectrophotometry (Table 2) were obtained.

It appeared of interest to examine cinnamon oil by the method for

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cassia oil, but, unfortunately, the presence of eugenol interferes in the region 1300 to 1200 cm^{-1} . Addition of 1.25% of *o*-methoxycinnamaldehyde to the oil did, however, introduce a detectable absorption at about 1260 cm^{-1} and it therefore appears unlikely that the methoxy-compound occurs in cinnamon oil.

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