# THE SPECTROPHOTOMETRIC DETERMINATION OF αβ-UNSATURATED ALDEHYDES AND KETONES WITH GIRARD-T REAGENT

## PART I. ESSENTIAL OILS

### BY J. B. STENLAKE AND W. D. WILLIAMS

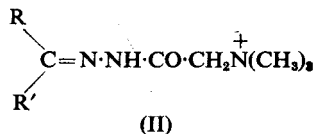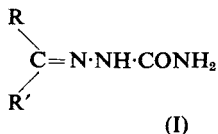*From The School of Pharmacy, The Royal College of Science and Technology, Glasgow*

Spectrophotometric methods for the determination of citral, cinnamaldehyde and carvone in essential oils using Girard-T reagent are described. The results are compared with those obtained by the hydroxylamine hydrochloride method.

REVIEWS of the determination of aldehydes and ketones in volatile oils[1-5] and other substances[6] are frequent and indicate the difficulties inherent in the assays. Unless a reagent can be made almost specific for one type of carbonyl compound, for example m-phenylenediamine dihydrochloride for the αβ-unsaturated group[7], chemical methods do not distinguish between different types and the result is necessarily expressed in terms of the major component such as citral in lemon oils. This account is concerned with the development of a rapid spectrophotometric method for the determination of the αβ-unsaturated carbonyl compounds citral, cinnamaldehyde and carvone in volatile oils. The above mentioned m-phenylenediamine colour reaction[7] has been used for the determination of αβ-unsaturated aldehydes in oils, but it does not react with carvone, and, moreover, suffers from the disadvantage that the colours fade rapidly. Measurement of the general ultra-violet absorption of Californian and Italian lemon oils has been used[8,9] as a criterion of purity, but the high probability of irrelevant absorption in the appropriate wavelength region renders the method of doubtful value for quantitative work. Carvone has, however, been determined by means of the high intensity αβ-unsaturated carbonyl absorption[10] a carvone-free oil being used to compensate for the absorption by terpenes and other constituents. Interference due to irrelevant absorption can be overcome by conversion of the carbonyl compounds to the corresponding semicarbazones and 2:4-dinitrophenylhydrazones which exhibit characteristic high intensity absorption at longer wavelengths than those associated with the parent compounds. Such derivatives however still require organic solvents for solution, and the volatile oils would of necessity be retained in the solution undergoing measurement. This latter disadvantage can, however, be overcome by the use of Girard-T reagent, the derivatives of which are water-soluble and readily separated from non-carbonyl constituents of the oil. Like the semicarbazones Girard-T hydrazones of αβ-unsaturated carbonyl compounds show considerably greater absorption than the parent substances. This, together with the longer wavelength of the maxima, not only tends to reduce errors caused by irrelevant absorption, but also increases the sensitivity of the method by a factor of about 2·5.

Girard reagents[11] have been used extensively for the isolation of aldehydes and ketones from natural products and reviews appear occasionally on this subject[12,13]. For the quantitative determination of the hydrazones polarography[14-19] has proved a useful tool, but ultra-violet absorption has received little attention. Höyer[20] noted the similarity of the chromophore in semicarbazones (1) and Girard-T hydrazones (II).

$$\underset{R'}{\overset{R}{>}}C=N \cdot NH \cdot CONH_2 \qquad\qquad \underset{R'}{\overset{R}{>}}C=N \cdot NH \cdot CO \cdot CH_2 \overset{+}{N}(CH_3)_3$$

$$(I) \qquad\qquad\qquad\qquad (II)$$

and made use of the ultra-violet absorption to determine αβ-unsaturated ketosteroids in urine. Spectrophotometry was also used by Young[21] to confirm the formation of Girard-T derivatives of some saturated ketones. For quantitative work, however, the absorption of saturated derivatives, although intense at 230 to 240 mμ is of less value because of the increase in the absorption by the reagent in this region. Ideally, the maximum absorption should occur at a wavelength greater than about 265 mμ above which there is little interference. The Girard-T hydrazones of αβ-unsaturated carbonyl compounds comply with this condition.

## DEVELOPMENT OF METHOD

*Standards*

Cinnamaldehyde yielded a crystalline Girard-T derivate m.p. 175° $E$ (1 per cent, 1 cm.) at 313 mμ = 1492, but the poor analytical figures and its hygroscopic nature confirmed its unsuitability as an analytical standard. We therefore used pure cinnamaldehyde as standard, which *when treated by the given method*, gave an $E$ (1 per cent, 1 cm.) value equivalent to that obtained with the crystalline Girard-T hydrazone. Thus despite our inability to isolate the corresponding crystalline Girard-T derivatives from citral and carvone we consider that *pure citral* and *pure carvone* provide satisfactory standards.

Pure citral was prepared by Tiemann's method as modified by Hibbert and Cannon[22]. The product is a mixture of citral-*a* and citral-*b*. As the ultra-violet absorption spectra of the corresponding semicarbazones are identical, it is assumed that those of the Girard-T hydrazones are also identical.

Carvone of satisfactory physical constants was prepared from dill oil through the semicarbazone which was isolated in the form m.p. 140 to 141°.

Typical absorption curves for reagent and the Girard-T hydrazones of citral, cinnamaldehyde and carvone are given in Figure 1. The molecular extinction coefficients of the hydrazones and those published for the corresponding semicarbazones (Table I) are similar. The bathochromic shift of the absorption maximum is attributed to solvent effect. This comparison provides support for accepting the curves obtained with citral, carvone and cinnamaldehyde (Fig. 1) as being due to the hydrazones although pure crystals of these hydrazones were not isolated. Derivatives

prepared from natural oils showed no variation in the wavelengths of the maxima (Table II).

We have shown (Fig. 2) that the absorption of all three compounds obeys the Beer-Lambert Law, and this eliminates the need for calibration curves in the methods described.
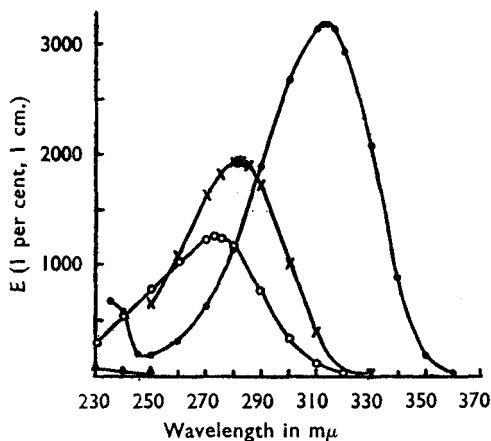


FIG. 1. Absorption spectra of citral ($\times$), cinnamaldehyde ($\bullet$) and carvone ($\bigcirc$) after the treatment described under the appropriate method of assay. Girard-T reagent ($\blacktriangle$).
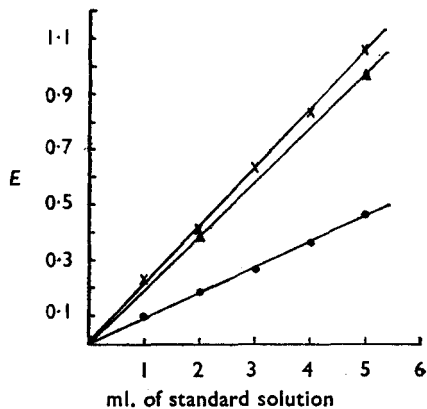
FIG. 2. Calibration curves for citral ($\times$) 0·559 per cent w/v in ethanol; cinnamaldehyde ($\blacktriangle$) 0·366 per cent w/v in ethanol; carvone ($\bullet$) 0·770 per cent w/v in ethanol.

*Reaction Solvents*

Owing to the different rates at which aldehydes and ketones react with Girard-T reagent, different solvents were found to be necessary. Citral and cinnamaldehyde reacted quantitatively in ethanol, in agreement with the observations of Lederer and Nachmias[26]. Carvone required the solvent proposed by Girard and Sandulesco[11] which is a 10 per cent solution of glacial acetic acid in ethanol. Lemon oils, which contain only small quantities of carbonyl compounds, modify the solvent system appreciably and appear to slow the reaction. The decrease in the $E$ (1 per cent, 1 cm.) value was seen in preliminary control experiments with solution of citral in oil of turpentine. The difficulty can be overcome by increasing the quantity of reagent and limiting the amount of essential oil. On the other hand the concentration of carbonyl compounds in cinnamon, dill and caraway oils is very much higher and in consequence the oil sample is much smaller so that the effect of terpenes in such materials was neglected. $E$ (1 per cent, 1 cm.) values found in this series are lower than the standard figure for citral even when turpentine was absent. Satisfactory results were obtained only when the usual 30 seconds drainage time for pipetting ethanolic solutions was extended to 80 seconds for the citral solution at which time the pipette delivered exactly one tenth of the quantity of solution in a 50 ml. standard flask. Complete concordance was then found between results obtained by direct weighing of samples and those in

TABLE I

MOLECULAR EXTINCTION COEFFICIENTS OF GIRARD-T HYDRAZONES AND SEMICARBAZONES
OF CITRAL, CINNAMALDEHYDE AND CARBONE

| Compound | Girard-T hydrazone | | Semicarbazone | | Ref. |
|---|---|---|---|---|---|
| | $\epsilon$ | λ max. (mμ) | $\epsilon$ | λ max. (mμ) | |
| Citral .. .. .. | 29,500 | 281 | 31,350 | 272 | 23 |
| Cinnamaldehyde .. | 42,000 | 313 | 40,200 | 310 | 24 |
| Carvone .. .. | 19,000 | 272·5 | 21,200 | 265 | 25 |

which a weighed quantity had been diluted and an aliquot portion taken by volume.

*Reaction Time*

Using a twofold excess of the reagent, reaction with citral and cinnamaldehyde in ethanol was complete in 10 minutes, and a standard time of 12 minutes was therefore chosen. As expected carvone was slow to react even in acetic acid-ethanol. Evaporation of the ether remaining after the extraction of the carvone Girard-T hydrazone yielded traces of oil when natural oils were examined. These residues gave positive reactions with 2 : 4-dinitrophenylhydrazine when less than 60 minutes were given for the reactions, confirming that the reactions were incomplete. We have confirmed spectrophotometrically that the reaction was complete in 60 minutes (Fig. 3) and a standard time of 70 minutes was adopted for carvone.

TABLE II

| Oil | Quantity (mg.) | Dilution (ml.) | λ max. (mμ) | $E$ (1 per cent, 1 cm.) |
|---|---|---|---|---|
| Citral .. .. .. | 10–15 | 10 to 100 | 281 | 1937 |
| Lemon .. .. | 300 | 10 ,, 100 | 281 | |
| Lemon grass .. .. | 15–20 | 10 ,, 100 | 281 | |
| Cinnamaldehyde .. | 10–15 | 10 ,, 250 | 313 | 3180 |
| Cinnamon bark .. | 20–30 | 10 ,, 250 | 313 | |
| Cinnamon leaf .. | 200 | 10 ,, 250 | 313 | |
| Carvone .. .. | 15–20 | 10 ,, 100 | 272·5 | 1267 |
| Dill .. .. .. | 20 | 10 ,, 100 | 272·5 | |
| Caraway .. .. | 20 | 10 ,, 100 | 272·5 | |

*Stability of Derivatives*

Girard-T hydrazones regenerate the parent carbonyl compounds under acid-aqueous conditions, and as the final solutions in some of the assays were of pH 5·1 to 5·3, the optical density was checked at intervals to detect possible dissociation of the complex. No change in the optical density was observed over many hours.

*Calculation of Results*

Since the observed absorption is strictly proportional to concentration, results can be calculated by using the $E$(1 per cent, 1 cm.) values given in Table II. These values are referred to the parent carbonyl compound *when treated by the given method* and are not the $E$(1 per cent, 1 cm.) values for the corresponding Girard-T hydrazones.

*Recovery Experiments with Citral*

The quantitative nature of the method finally adopted for the determination of citral in essential oils was demonstrated by a series of recovery experiments on mixtures of pure citral and oil of turpentine. There was prepared 4·93, 4·11, 3·30 and 3·55 per cent w/w; found respectively, 4·95 (100·4), 4·11 (100), 3·34 (101·2) and 3·53 (99·5). Per cent recoveries are given in parentheses.
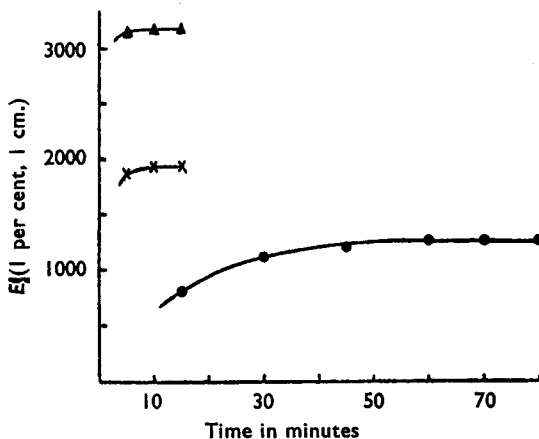
EXPERIMENTAL

Melting points are uncorrected. Ultraviolet absorption spectra were measured in 1 cm. cells using a Hilger Uvispek spectrophotometer, model H700/303.



FIG. 3. Rates of reaction of citral (×), cinnamaldehyde (▲) and carvone (●) with Girard-T reagent in an appropriate solvent.

*Preparation of Standards. Citral.* Commercial citral (49 g.) was added to a cooled solution of sodium sulphite (175 g.) and sodium bicarbonate (62·5 g.) in water (500 ml.), and the mixture was shaken vigorously for one hour. The oily phase was extracted with ether (2 × 300 ml.) and rejected. The aqueous phase was treated with 10 per cent sodium hydroxide solution until the solution became turbid and was extracted immediately with ether (2 × 300 ml.). The bulked ether layers were washed with tartaric acid solution until the washings were acid to litmus, dried with sodium sulphate and evaporated. The residue was fractionally distilled and the fraction (25·2 g.) b.p. 118°/16 mm. reserved as standard. The first runnings (4·5 g.) and residue (5·1 g.) were rejected. The standard was an almost colourless oil $n_D^{16·5°}$ 1·4907, $d_{20}^{20}$ 0·8895, $E$(1 per cent, 1 cm.) at 281 m$\mu$ = 1937 when treated by the method given below.

*Cinnamaldehyde Girard-T hydrazone.* Cinnamon oil (4 g.) was treated with Girard-T reagent (3 g.) in ethanol (10 ml.) and heated on a boiling water bath for 30 minutes. The crystalline product which separated on slow cooling was filtered off and recrystallised from ethanol as pale yellow needles, m.p. 175° (decomp.) $E$(1 per cent, 1 cm.) at 313 m$\mu$ = 1492.

*Cinnamaldehyde.* Cinnamaldehyde of reagent grade was distilled under reduced pressure and the pale yellow fraction b.p. 134°/22 mm. $d_4^{17°}$ 1·049, $n_D^{17°}$ 1·6215, used as standard.

*Carvone.* Oil of dill (22 g.) was heated for 1½ hours with semicarbazide hydrochloride (15 g.), sodium acetate (anhydrous, 12 g.) and ethanol (90 per cent, 200 ml.). The mixture was cooled, diluted with water (100 ml.) and set aside until the turbid liquid deposited a bulky mass of

crystals. The semicarbazone was washed free from oil, and recrystallised from ethanol as colourless flat needles (9 g.) m.p. 140 to 141°. The crystals were decomposed by addition of phthalic acid (3 g.), and steam-distillation until 1200 ml. of distillate had been collected. The carvone was isolated from the distillate by ether extraction, drying with sodium sulphate and evaporation of the ether. Distillation of the residue under reduced pressure yielded a colourless oil (3·5 g.) b.p. (bath temp.) 120°/18 mm. $n_D^{20°}$ 1,5000, $d_{18°}^{18°}$ 0·9625, $[\alpha]_{18°}$ +61·7° (C = 2·2 in ethanol), $E$(1 per cent, 1 cm.) 272·5 m$\mu$ = 1267 when treated by the method given below.

### Reagents

Girard-T reagent recrystallised twice from ethanol and stored over sulphuric acid; ethanol (absolute); solvent ether; glacial acetic acid (A.R.); N sodium hydroxide.

### Method for the Determination of Citral and Cinnamaldehyde in Essential Oils

Boil an accurately weighed quantity of the oil (see Table II for the appropriate quantity)* for 12 minutes under reflux with ethanol (5 ml.) and Girard-T reagent (0·2 g.). Cool the mixture and transfer to a separator with the aid of *ether* (50 ml.). Wash out the reaction flask with water (25 ml.) and transfer the washings to the separator. Shake the mixture gently, allow to separate and run the lower aqueous layer into a 500 ml. graduated flask. Wash the reaction flask and ether layer with two further quantities of water, each of 10 ml., and add the washings to the flask. Adjust to 500 ml. with water, mix well and dilute an aliquot portion so as to obtain a suitable optical density for measurement in 1 cm. cells at the wavelength of peak absorption (see Table II for the appropriate dilution). Carry out a blank determination without the oil and use as reference solution in matched cells. Calculate the percentage of carbonyl compound by means of the given $E$(1 per cent, 1 cm.) constants (Table II).

### Preliminary Experiments with Carvone

(i) Carvone, in ethanol, treated with Girard-T reagent as described under the Method for the Determination of Citral in Essential Oils did not react.

(ii) Addition of glacial acetic acid (10 per cent v/v) to the reaction mixture promoted reaction which was complete in 60 minutes (Fig. 3).

### Method for the Determination of Carvone in Essential Oils

Boil an accurately weighed quantity of oil (see Table II for the appropriate quantity) for 70 minutes under reflux with ethanol (5 ml.), glacial acetic acid (0·5 ml.), and Girard-T reagent (0·2 g.). Cool the mixture and transfer, with the aid of ether (50 ml.), to a separator which contains

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* For small quantities the most convenient method is to dilute the oil with ethanol so that 5 ml. of the dilution contains the required amount.

sufficient N sodium hydroxide to neutralise 9/10 of the acetic acid. Complete the assay as described under the Method for the Determination of Citral in Essential Oils.

## RESULTS AND DISCUSSION

The well-known hydroxylamine hydrochloride method[27] determines total aldehydes or ketones in volatile oils and it appeared of interest to compare the results with those obtained by the spectrophotometric method (Table III). The latter method is specific for $\alpha\beta$-unsaturated compounds so that citronellal and methylheptenone in lemon oils would not be estimated.

### TABLE III

RESULTS OF SPECTROPHOTOMETRIC AND CHEMICAL METHODS OF EXAMINATION OF ESSENTIAL OILS

| Oil | | | | Found (per cent w/w) | |
|---|---|---|---|---|---|
| | | | | Citral | |
| | | | | Girard-T | Chemical |
| Standard citral (re-examination) | .. | .. | | 99·3* | 98·7* |
| Commercial citral A | .. | .. | .. | 90·1 | — |
| ,, ,, B | .. | .. | .. | 93·2 | — |
| ,, ,, B* | .. | .. | .. | 92·2* | 92·7* |
| Lemon .. .. C | .. | .. | .. | 3·70 : 3·72 3·74 : 3·73 3·73 | 4·1† |
| ,, .. .. D | .. | .. | .. | 3·55 | 4·3† |
| ,, .. .. E | .. | .. | .. | 3·77 | 4·5† |
| Lemon grass .. F | .. | .. | .. | 66·9 | 69·8 |
| ,, ,, .. G | .. | .. | .. | 67·3 | 71·3 |
| | | | | Cinnamaldehyde | |
| Cinnamaldehyde, reagent grade | .. | .. | | 97·7 | 97 |
| Cinnamaldehyde (from bisulphite comp.) | | .. | | 99·8 | — |
| Cinnamon bark H | .. | .. | .. | 63·4 | 64·5† |
| ,, ,, I | .. | .. | .. | 62·5 | 61·9† |
| ,, ,, J | .. | .. | .. | 64·1 | 64·0† |
| ,, ,, K | .. | .. | .. | 58·3 : 58·9 | 59·8 |
| ,, leaf L | .. | .. | .. | 1·67 : 1·66 | 2 (approx.) |
| | | | | Carvone | |
| Carvone (synthetic), reagent grade | .. | .. | | 95·0 | 97·5 |
| Carvone (from semicarb.) | .. | .. | | 100·0 | 101 |
| Caraway .. M | .. | .. | .. | 54·3 : 54·3 | 54·8 |
| ,, .. N | .. | .. | .. | 49·4 : 49·1 | 55 approx.) |
| Dill .. | .. | .. | .. | 45·7 | 53 ( ,, ) |

* Assays after six week interval in which visible deterioration had occurred in the standard citral.
† Chemical analysis by Stafford Allen and Sons Ltd.

The differences found were so large (Table III) and raised such doubts in our results that the standard and the commercial citral (B) were re-examined by both methods. Satisfactory agreement was obtained so that differences in the figures for lemon oils themselves by the two methods can only be attributed to the presence of other carbonyl compounds. The slightly lower figures obtained for citral on re-examination were probably caused by deterioration, since the standard had acquired a distinct yellow colour during the interval of six weeks between assays. Before a final conclusion can be reached on natural lemon oils a larger number of samples must be examined.

Cinnamon oils may contain o-methoxycinnamaldehyde which would be calculated as cinnamaldehyde by the spectrophotometric method. Closer agreement between the results by both methods was expected and actually found (Table III); cinnamon leaf oil (L) proved very difficult to assay

chemically because of the small cinnamaldehyde content so that an approximate result only was obtained. No difficulty was experienced when using Girard-T reagent.

Dihydrocarvone is likely to occur with carvone in caraway and dill oils but agreement was obtained between both methods for a genuine sample of English caraway oil (Table III M). The samples of caraway (N) and dill oils are of interest. Both were discoloured and of considerable age but examination by the official method indicated a satisfactory carvone content. The endpoint in both assays however was doubtful as a pink tint persisted and increased in intensity on the addition of a large excess of alkali. As the spectrophotometric method on the caraway oil gave results which did not comply with the British Pharmacopoeia requirements, the physical constants of the oil were determined. These were satisfactory except for the optical rotation ($+64°$) which is outside the official limits. It therefore affords a measure of support for the spectrophotometric result.

The results so far obtained indicate the utility of the Girard-T reagent, particularly where only small quantities of aldehyde occur for example in cinnamon leaf oils. The method provides a closer approximation to the true citral content of lemon oils though this may not necessarily be a guide to the organoleptic properties of these oils.

We wish to thank Dr. W. Mitchell of Stafford Allen and Sons Ltd. for gifts of essential oils and allowing us to include their results of several chemical assays. Thanks are also due to Miss E. Heggarty and Mr. W. Gardiner for their help.

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