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Note

The chemistry of terpenes

VII. New procedure for the determination of neral and geranial in essential oils*, **

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The determination of neral and geranial in essential oils presents many problems. The majority of analytical procedures which are currently available[3–16] (for a review, see ref. 3) are, in the main, non-specific or require the frequent construction of calibration curves using samples of pure citral which, due to its instability (see, *e.g.* ref. 17), must be freshly prepared. The only method which is generally accepted to be specific and accurate for the estimation of neral and geranial in the presence of other aldehydes is that described by Levi and Laughton[18] and involves the measurement of the electronic spectrum of the condensation product of neral and geranial with barbituric acid. Other "wet analysis" methods, for example, the formation of the bisulphite adduct or oxime[11–16], are non-specific and give anomalously high values (see Table I).

A full gas–liquid chromatographic (GLC) quantitative analysis of an essential oil is a relatively simple, although time consuming, procedure. However, even when precautions have been taken to minimize thermal and catalytic decomposition of the components, evaluation of the data using the internal normalization procedure usually gives anomalously high values for the neral/geranial content. This inherent limitation can be obviated by the use of an internal standard and the accuracy and reproducibility of the method then appear to be as good as that obtained using the procedure of Levi and Laughton. We have found, however, that it is advisable to examine the separation of the components of the essential oil on at least two stationary phases, as the elution times of neral and geranial are frequently close to those of other terpenoids such that the peaks overlap.

As an alternative to the simple GLC procedure, we have used a method based

* For Part VI, see ref. 1.
** Taken from the M.Sc. Thesis of M. E. Neale[2].
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upon the initial reduction of the neral and geranial in the essential oils with sodium borohydride, followed by GLC analysis of the nerol and geraniol. The procedure has the advantage that only neral and geranial are reduced to give nerol and geraniol, respectively, and that the elution times of the two alcohols are sufficiently different from other terpenoids on Carbowax 20M at 115° to permit direct analysis on a single column using an internal standard.

EXPERIMENTAL

The terpenoid samples were analysed using a Pye Series 105 chromatograph fitted with a 2.7 m × 4 mm (I.D.) glass column containing 2% (w/w) Carbowax 20M on Chromosorb W HP at 115° and a flame ionization detector at 200°. The injection temperature was 200° and the nitrogen carrier gas inlet pressure was 13.5 p.s.i. with a flow-rate of 45 ml/min.

Analytical procedure

A sample of the essential oil (100/x g, where x is the approximate percentage nerol/geraniol content of the oil) and the internal standard (2-phenylethanol) (0.6 ± 0.001 g) in methanol (25 ml) were heated under reflux with ethanolic potassium hydroxide (5 ml, 0.5 *M* in 90% v/v aqueous ethanol) for 30 min. The solution was cooled to room temperature and 0.2 µl of the supernatant liquid was injected directly on to the GLC column. The "natural nerol/geraniol" content was determined using the equation

$$\% \text{ (w/w) nerol/geraniol} = \frac{100 f m (A_1 + A_2)}{A_3}$$

where A_1, A_2, and A_3 are the peak areas for geraniol, nerol and 2-phenylethanol, respectively, m is the ratio (w/w) of the internal standard to the essential oil, and f is the correction factor for the differing detector response factors of nerol/geraniol and 2-phenylethanol, calculated from a quantitative analysis of a standard solution of 2-phenylethanol (0.6 ± 0.001 g) and Geraniol Intermediate B (Bush, Boake, Allen, London, Great Britain) (1.0 ± 0.001 g) in aqueous methanol (30 ml, 80% v/v).

A second sample of the essential oil (1.2 ± 0.001 g), the internal standard (2-phenylethanol) (0.6 ± 0.001 g) and sodium borohydride (0.3 g) in aqueous methanol (30 ml, 80% v/v) were heated under reflux for 30 min. The solution was allowed to cool to room temperature and 0.2 µl of the supernatant liquid was injected directly on to the GLC column.

The total nerol/geraniol content in the reduced sample was determined using the procedure described above and the percentage of neral/geranial in the essential oil was estimated as follows:

% (w/w) neral/geranial = 0.987 (% nerol/geraniol in reduced sample —
% nerol/geraniol in "hydrolysed sample)

The factor 0.987 corrects for the difference in the molecular weights of the aldehydes and alcohols.

RESULTS AND DISCUSSION

Comparison of the data presented in Table I shows that the currently used

TABLE I

PERCENTAGE (w/w) NERAL/GERANIAL CONTENT OF ESSENTIAL OILS BY DIFFERENT ANALYTICAL PROCEDURES

Sample	*Method*					
	*Bisulphite absorption**,**	*Oximination***,***	*GLC (internal normalisation)*	*Barbituric acid*§	*GLC (internal standard)*	*$NaBH_4$ reduction–GLC*§§
Lemongrass oil (synthetic)	70	74.4	74.2	64.9	63.5	60.7
Lemongrass oil (Cochin)	68	79.8	77.3	64.7	65.9	61.2
Lemongrass oil (Guatemala)	74	75.4	72.8	68.0	70.5	67.4
Leptosperum citratum oil	82	73.3	55.5	45.3	46.4	46.2
Litsea cubeba oil	74	76.2	76.2	66.1	69.3	67.3

* Total "citral" (v/v) content evaluated[3].
** Non-specific for neral/geranial.
*** Determined by the procedure described by Stillman and Reed[16].
§ Determined by the method described by Levi and Laughton[18]. Reproducibility ±0.9%.
§§ Reproducibility ±0.8%.

barbituric acid method gives considerably lower values for the neral/geranial content of the essential oils than the other less specific "wet analysis" methods. There is a close correspondence, however, between the barbituric acid method, the GLC internal standard method, and the procedure presented in this paper. Statistical examination of our procedure shows it to be reproducible (±0.8%). Nerol and geraniol were found to be completely inert towards sodium borohydride under the analytical conditions but errors could arise from the natural presence of nerol and geraniol in the essential oils and by the hydrolysis of their esters under the basic reduction conditions. Allowance for the total "natural nerol/geraniol" content was made by a separate analysis of the essential oils subsequent to the complete hydrolysis of the esters with ethanolic potassium hydroxide. Interference from the reduction of other aldehydes, *e.g.*, citronellal, was insignificant as the retention times of the alcohols formed were sufficiently different from those of nerol and geraniol on Carbowax 20M. This was not the case for the reduction of the three isocitral isomers[19], which gave two isomeric alcohols, one of which had an elution time on Carbowax 20M almost identical with that of nerol[2]. However, as the isocitral content of most essential oils is less than 2% of the citral content, we consider any inaccuracies from this source to be within experimental error.

GENERAL CONCLUSIONS

We consider that the new procedure described here for the determination of neral and geranial in essential oils is a sound alternative to the barbituric acid method. The method is specific, accurate, and reproducible and it does not require pure samples of citral for calibration.

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