Journal of Chromatography, 260 (1983) 173–176
Elsevier Science Publishers B.V., Amsterdam — Printed in the Netherlands

CHROM. 15,633

Note

Terpenoid analysis

II. The separation of some monoterpene alcohols by high-performance liquid chromatography with electrochemical detection

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(Received December 17th, 1982)

Monoterpenes are readily separable by chromatographic methods using either classical or high-performance liquid systems[1–3]. Whilst it has been shown[4] that terpenoids lacking really strong chromophores may be detected by UV in analytically useful concentrations, it would be preferable to have other detection methods available. To this end a number of monoterpene alcohols have been investigated by voltametric procedures. Thymol and carvacrol are readily detected by this procedure and the results are compared with those previously reported[4,5] using UV detection.

EXPERIMENTAL

The high-performance liquid chromatographic (HPLC) system was made from Spectra-Physics modules. An SP-8770 pump and injector system was used to deliver solvent to the 25-cm column packed with Spherisorb ODS (5 μm) (Technicol, Manchester, Great Britain). A Metrohm 641 wall-jet electrochemical detector produced a signal that was processed by a SP-4100 computing integrator. The detector had a conventional three-electrode configuration, which avoids non-linear responses as the current flow alters[6].

Water was double-distilled from all-glass apparatus and the other HPLC-grade solvents were obtained from Fisons. These were filtered and vacuum de-gassed before use. Other chemicals were supplied by Fluka. The flow-rate was set at 1.5 cm^3 min^{-1} and was monitored by a flow-meter manufactured by Phase Separations.

Scans of applied voltage *versus* current were recorded at 150 mV min^{-1} using a Tacussel Model EPL-1 polarographic system with Metrohm glassy-carbon and Ag/AgCl electrodes.

RESULTS AND DISCUSSION

In the wall-jet electrochemical flow cell, the electrode surface is continually swept with fresh solvent allowing reproducible signals to be obtained from replicate injections. The glassy-carbon electrode surface was cleaned with a fine abrasive paste

0021-9673/83/$03.00

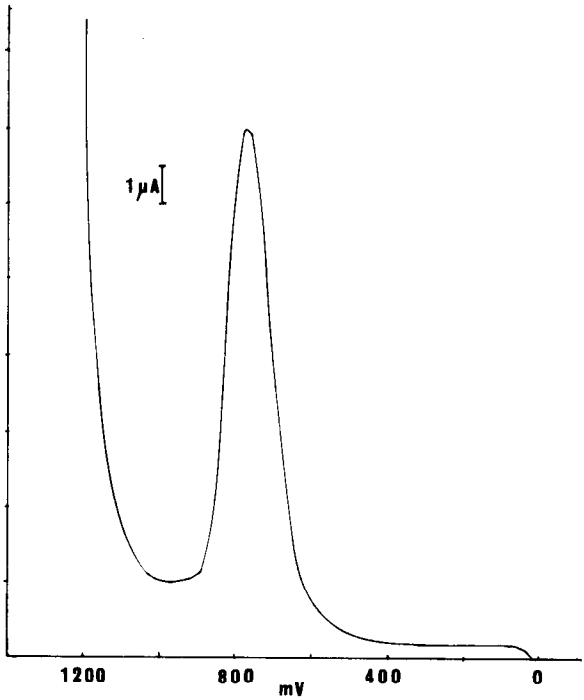

Fig. 1. Linear sweep voltammogram for thymol [0.5 mg cm^{-3} of acetonitrile–water (40:60) 2 g dm^{-3} lithium perchlorate as support electrolyte].

at the start of this work and was found to retain its characteristics over a period of at least 1 month without further attention. This was true even when using lithium perchlorate as the support electrolyte. The cell was thermostatted at 23°C and enclosed in a metal box that acted as an efficient shield to stray electrical signals. Absence of these facilities led to greatly increased noise levels and noise spikes. No appreciable dependance of noise to flow-rate was observed when testing the system with various trial substances (*e.g.*, noradrenaline) and so a relatively high flow-rate of 1.5 cm^3 min^{-1} was used. This contrasts with a recent report[7] where flows of around 0.5 cm^3 min^{-1} were commended.

It was found to be most economic of time to carry out initial trials with a polarographic apparatus. This was modified to take a glassy-carbon electrode instead of the normal dropping-mercury type. Linear sweep voltammograms could then be used to assess the detection possibility of the various monoterpene alcohols. In contrast to flow operations the electrode had to be carefully cleaned before each determination. Despite this necessity a scan of this type produces more useful information for method development than is possible from running many chromatograms at different (fixed) applied potentials. It was thus possible to assess the usefulness of different support electrolytes and their optimum concentration as well as deciding which alcohols could give responses. Of the monoterpene alcohols studied (carvacrol, geraniol, linalool, nerol, terpineol and thymol) only the phenolic species carvacrol and thymol gave useful traces (Fig. 1). Terpineol does show signs of oxidation (Fig. 2)

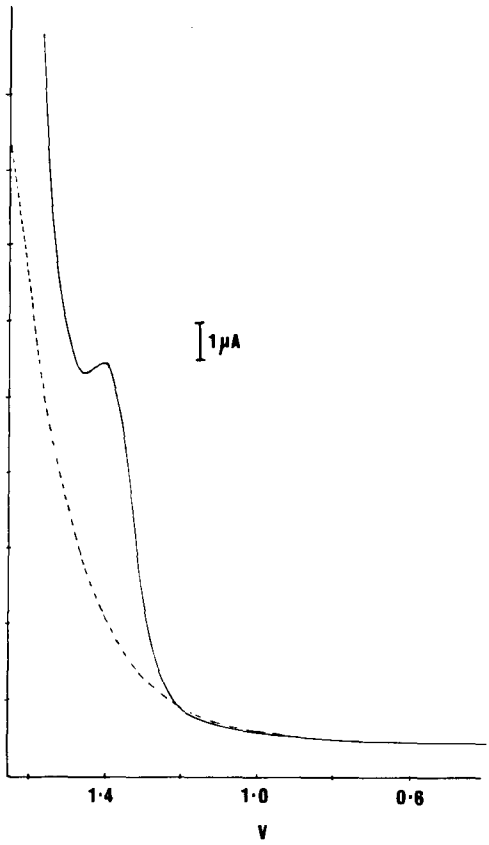

Fig. 2. Linear sweep voltammogram for terpineol (full curve) and solvent alone (dotted curve). [0.6 mg cm^{-3} of terpineol in acetonitrile–water (40:60) + 2 g dm^{-3} lithium perchlorate].

but at a working potential of 1.4 V against the Ag/AgCl reference electrode it is at the limit of usefulness of the glassy-carbon system. Sensitivity was only slightly affected by the support electrolyte and the choice of 2 g dm^{-3} of lithium perchlorate was made after comparison of peak heights and noise levels obtained with other salts (such as potassium nitrate and ammonium sulphate).

This electrochemical procedure offers great selectivity for the determination of phenolics in plant materials. Alcohols of different structures can be ignored if the applied potential is adjusted to a value of around 800 mV. This should be of great utility in such analysis as that reported[5] on the essential oil derived from *Thymus capitatus*. Using the same solvent system [acetonitrile water (40:60), with the addition of lithium perchlorate] good separations of thymol and carvacrol were obtained (Fig. 3). The response was assessed by both peak-heights and area. Slightly better results were obtained with the latter procedure. Response graphs were constructed from multiple injections of about six different concentrations. Simple regression analysis showed excellent linearity for both substances with standard deviations of 0.91 and 0.81% for carvacrol and thymol, respectively. This is well within the error range expected for a sample loop injector system.

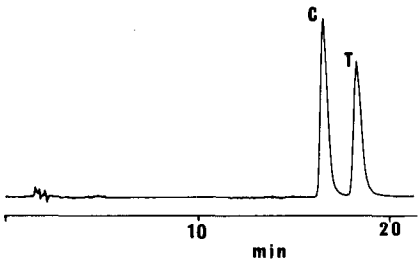

Fig. 3. HPLC trace for the separation of carvacrol (C) from thymol (T). Solvent: acetonitrile–water (40:60) + 2 g dm^{-3} lithium perchlorate. Electrochemical detector set at 800 mV.

A measurement of the noise level of the detector was made by pumping solvent through the column and monitoring the detector on the most sensitive ranges. This information was used to evaluate the system detection limit, taking the definition[8] as being that quantity of material required to produce a signal equal to the noise level. By this criterion the detection limits are 10 ng for carvacrol and 14 ng for thymol. These are only slightly better than those reported[4] by UV detection at 220 nm, where values of 20 ng were obtained.

Thus the electrochemical system offers no major improvement in sensitivity but is shown to have some value in selectivity in that fewer species give a response at the potentials employed.

ACKNOWLEDGEMENT

M. P. L. de H. acknowledges the support of a scholarship from the Universidad de Oriente (Venezuela).

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