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METHOD FOR RADIO-CAPILLARY GAS CHROMATOGRAPHY EMPLOYING A MODIFIED OXIDATION–REDUCTION TRAIN AND FLOW-THROUGH DETECTOR

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SUMMARY

A conventional radioactivity monitor was modified by reducing the volume of the oxidation–reduction train and gas proportional counting tube to permit coupled radio-capillary gas chromatographic analysis of labeled isomeric metabolites. The utility of this radioactivity monitoring technique was demonstrated by separating ^{3}H-labeled sesquiterpene olefins. The advantages and limitations of mass (thermal conductivity) and radioactivity detection by this method are discussed.

INTRODUCTION

Advances in gas–liquid chromatography employing fused-silica capillary columns and on-column injection techniques have extended both resolution and sensitivity for the analysis of volatile compounds. Methods for radio-gas chromatography[1] have not kept pace with capillary technology, as most commercially available radioactivity monitors are designed for packed-column use. Techniques that have so far been described for radio-capillary chromatography either lack the sensitivity needed to measure ^{3}H (by using a solid scintillator) or are limited in resolution (owing to dilution of the gas stream in a proportional counting tube)[2–4]. In this work, the configuration of a commercial radioactivity monitor (gas proportional counter) was simply modified to permit the use of capillary chromatography and thus take advantage of rapid, high-resolution separations of complex mixtures of labeled metabolites.

EXPERIMENTAL

Equipment

A Gow-Mac 550P gas chromatograph, equipped with a low-volume thermal conductivity detector (10-952) and dual WX7 filaments, was employed. Make-up gas (helium) was introduced through a precision needle valve prior to the detector via a simple tee-connector to which an Alltech Superox-FA column (30 m × 0.53 mm I.D., 1-μm bonded phase) was also joined. The inlet port was fitted with a direct-flash

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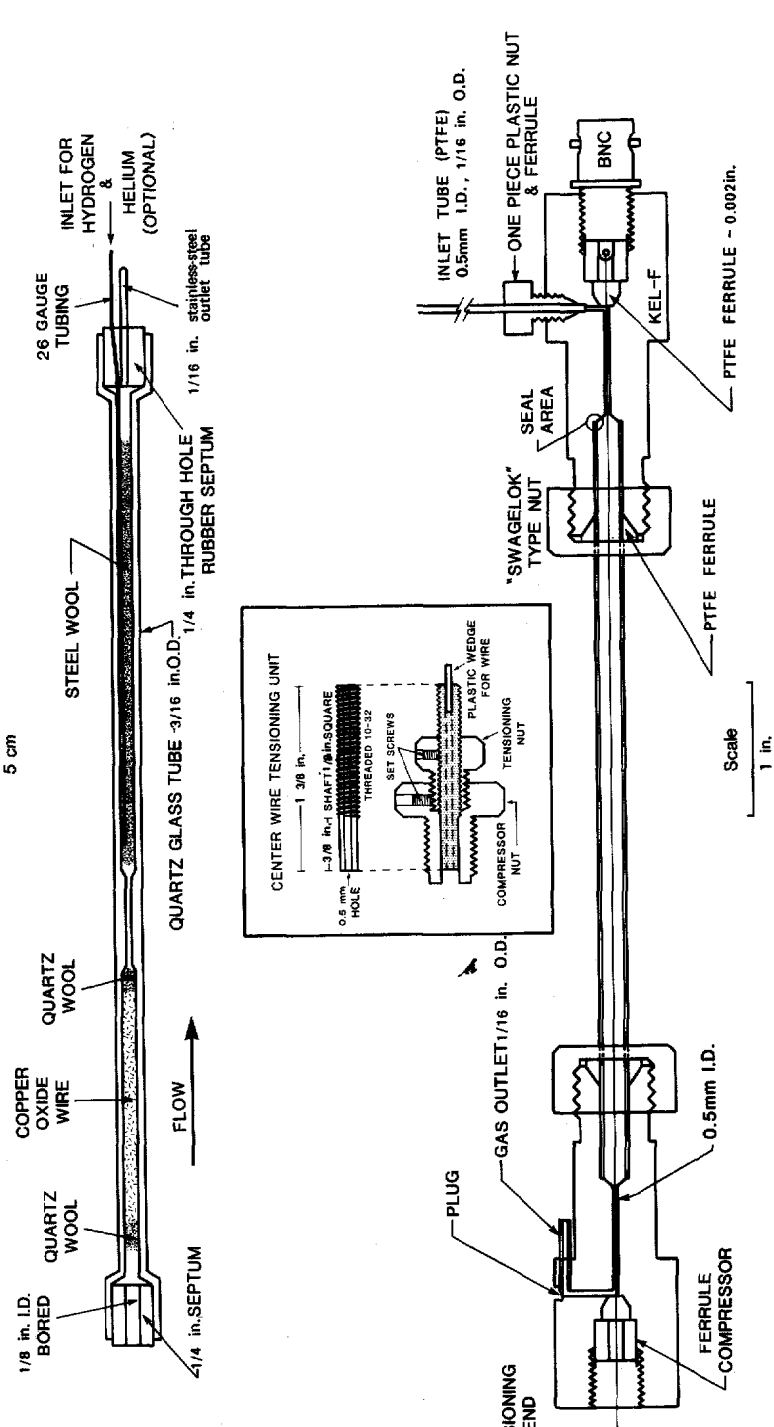

Fig. 1. Schematic diagram of modified oxidation–reduction train (above), gas proportional counting tube (below) and center wire tensioning unit. The ends of the oxidation–reduction train protrude outside of the furnace to prevent decomposition of the silicone-rubber septa. Fittings on the counting tube are non-conductive, with the exception of the stainless-steel central shaft used to tension the tungsten center wire. The stainless-steel counting tube is grounded to the instrument chassis.

injection liner (J & W 210-1064). The column flow-rate was set to 6 ml/min of helium via the inlet pressure gauge, with 6 ml/min of helium as make-up. The detector effluent left the cell in 1/8-in. stainless-steel tubing and was passed directly through a bored 1/4-in. through-hole septum into the combustion tube of the radioactivity monitor without need for a heated transfer line.

The radioactivitty monitor was a Packard 894 gas proportional counter that was modified as described below.

Combustion tube and counting tube modification

The combustion tube consisted of a 30 cm × 2.5 mm I.D. quartz tube, flared at both ends to accommodate 1/4-in. through-hole septa, with a 3-cm long central portion of 1 mm I.D. to separate the oxidizing and reducing components (Fig. 1). The inlet side (oxidizing) was packed with copper(II) oxide and a quartz-wool plug, and the outlet side (reducing) was packed with 4-0 steel wool (prewashed with chloroform–methanol (2:1, v/v)). No alteration to the aligned heating ovens was required. Hydrogen was introduced (<0.5 ml/min is sufficient to maintain iron in the reduced state under these conditions)[5] through the reducing end of the combustion tube via 26-gauge hypodermic needle tubing passed through the 1/4-in. septum and extending to the 1-mm restriction (additional make-up gas could be added by this means, if necessary). The combustion tube (preconditioned at 750°C under a flow of helium) was joined via 40 cm of 0.5 mm I.D. (1/16 in. O.D.) PTFE tubing to a drying tube consisting of 4 mm I.D. Pyrex tubing (15 cm) packed with 0.6 g of magnesium perchlorate and 1.2 g of carbon dioxide adsorbent (Mallcosorb; Mallinckrodt), if desired (these should be renewed daily when in heavy use).

A similar section of tubing connected the gas stream from the drying tube to the counting tube consisting of a 15-cm section of 4.1 mm I.D. (6 mm O.D.) precision-bore, polished stainless-steel high-performance liquid chromatographic tubing with inlet and outlet, and central high-voltage wire, configured essentially as in the original equipment (Fig. 1). The quench gas (propane) was regulated with the original valve of the proportional counter using a 5-m length of 0.05 mm I.D. restrictor capillary tubing to obtain a constant flow through the counting tube of 0.8 ml/min at 3 p.s.i. With an internal volume of about 2 ml and a total flow-rate of 12.5 ml/min, a residence counting time of approximately 10 s was achieved. A 6-ml counting tube was also constructed and tested, but offered no significant advantage.

Sample analysis

Labeled sesquiterpene olefins were prepared biosynthetically using cell-free extracts of *Salvia officinalis* (garden sage) as described previously[6]. The substrate used was [1-^{3}H]farnesyl pyrophosphate (90 Ci/mol)[7], and the labeled products were extracted from the reaction mixture with pentane, diluted with unlabeled carriers to *ca.* 3 Ci/mol and concentrated for analysis as described[6,7].

RESULTS AND DISCUSSION

Design considerations

In most designs for radio-gas chromatography, the ^{3}H- or ^{14}C-labeled sample components leave a non-destructive detector (*e.g.*, thermal conductivity) in an inert

gas and pass through a short, heated transfer line into a heated quartz "combustion" tube where each component is first oxidized to carbon dioxide and water over copper-(II) oxide, and then passed into a reducing environment over an iron catalyst (fine steel wool) to effect the reduction of water to hydrogen[3,5,8]. Residual water (if any) in the gas stream can be trapped on magnesium perchlorate, and the resulting $^{14}CO_2$ and $^{3}H_2$ are passed into the gas proportional counting tube. The $^{14}CO_2$ can also be trapped to permit the counting of only ^{3}H.

Capillary chromatographic detectors are designed with low "dead volumes" to maximize sensitivity and resolution. However, most conventional gas proportional counting tubes have a nominal internal volume of 20 ml, which is compatible with the flow-rates of 40–120 ml/min of helium used for packed columns. To employ such a monitoring system in capillary applications requires the addition of make-up gas to the optimum column flow-rate of *ca.* 6 ml/min to maintain in the counting tube the resolution achieved on the column, but such dilution of the labeled sample leads to peak distortion and severely reduces the sensitivity, which is inversely proportional to total flow-rate[9]. It was reasoned that, by employing both a combustion tube and counting tube of lower volume, the make-up gas volume could be reduced while maintaining resolution and improving sensitivity. The latter is, nevertheless, still dependent on the inherent efficiency of the counting tube configuration, and in this application is limited by the sample residence time rather than by gas stream dilution[9]. In practical terms, analysis is restricted to samples of moderate specific radioactivity (>1 Ci/mol). Such specific activities are easily achieved in biosynthetic experiments with labeled precursors at or above 30 Ci/mol, assuming 10- to 30-fold dilution with unlabeled carrier to permit sample manipulation. Rather, the major consideration in such experiments is often the need to resolve a complex mixture of metabolites, only some of which may be labeled.

To examine the operational limitations of such a capillary gas chromatograph–radioactivity monitor system, a Packard 894 gas proportional counter was modified, as described under Experimental, by reducing the internal volume of both the sample combustion–reduction train and the gas proportional counting tube, and by adding the auxiliary plumbing needed to provide make-up gas at the thermal conductivity detector inlet and to allow operation of the system at relatively low flow-rates of carrier, hydrogen and quench gas.

Sample analysis

To test the system, a series of ^{3}H-labeled sesquiterpene olefins were analyzed. These compounds are of moderate volatility, and it is often difficult to separate the complex mixtures of these metabolites produced *in vivo* from labeled precursors or in cell-free extracts. Fig. 2 illustrates a typical separation of sesquiterpene olefin isomers ($C_{15}H_{24}$) in which the mass and radioactivity detector outputs were monitored simultaneously with a SICA 7000A processor. The slight delay between the thermal conductivity detector trace and radioactivity signal is due to the transit time between the detector and the counting tube. However, the dual-channel integrator allows straightforward peak correlation and simple external calibration with [^{3}H]toluene or other labeled standards (whereas such relative calibration is of greatest practical value, the absolute counting efficiency can be calculated from the peak area, sample dpm and counter residence time). The detection limit with this system is about 2×10^{3} dpm per

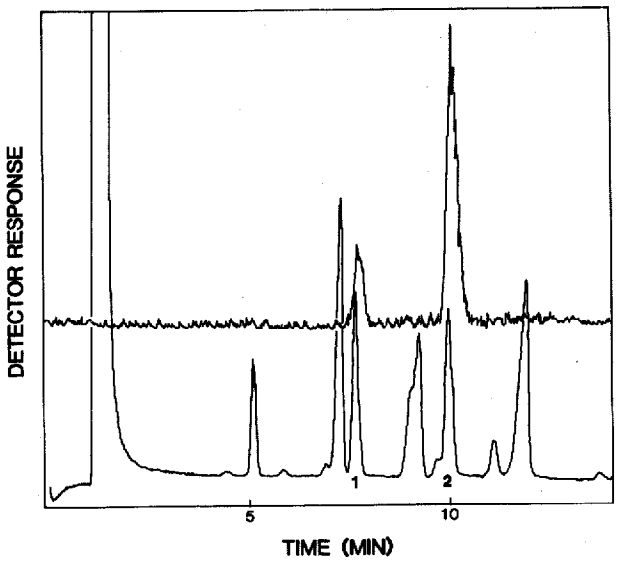

Fig. 2. Radio-capillary gas chromatographic separation of ^{3}H-labeled sesquiterpene olefins. The lower trace is the thermal conductivity detector response (at 4 × attenuation) to a mixture of olefins consisting of α-, β- and γ-patchoulene, α-guaiene, caryophyllene, α-bulnesene, humulene, β-elemene, gurgunene and seychellene. The sample (1 μl of hexane containing 20 μg of total olefins and about 10^4 dpm of ^{3}H) was separated at 140°C on Superox-FA under the conditions described in the text. The upper trace (5000 dpm full-scale setting) illustrates the radioactivity response of the modified gas proportional counter coupled directly to the chromatograph. Only caryophyllene (1) and humulene (2) were shown to be labeled.

component and roughly 30 ng per component, with an optimum of 10^4–10^5 dpm per 0.1–5 μg. The limit of resolution in radiochemical separation is about 20 s, peak-to-peak, with the 2-ml counting tube and a gas flow-rate of 12 ml/min. As the resolution in the chromatographic separation is nearly three times that of the radiomonitor, some overloading is permitted, provided that peak skewing is within the 20-s resolving range of the monitor. An additional advantage of the capillary system is the reduction of variations in flow-rate with temperature changes, relative to packed-column operation. The net result is a slower rate of baseline drift at the thermal conductivity detector, and a more uniform counting efficiency due to a more stable quench gas to carrier gas ratio. The low flow settings of hydrogen, carrier and quench gases were adequately managed with conventional regulators (Alltech, Cat. No. 19 992) and flow restrictors, although higher quality flow controllers would improve the performance, particularly with temperature programming.

The commercial gas proportional counting tube operates routinely in the 1700–1800 V range, whereas the tube of lower volume operates with optimum sensitivity at 1400–1500 V, with a narrower voltage "plateau" at which proportional counting is obtained. The small-volume tube is also more prone to discharge (arcing) on introduction of the solvent, but this difficulty is easily avoided by delaying the application of high voltage to the system until the solvent peak has passed (the system recovery time is <1 s following voltage application). Other practical considerations include the introduction of hydrogen in the reduction train to maintain the iron

catalyst in a reduced state (and to assist in purging the $^3H_2$ produced). The lifetime of the combustion tube is then largely dependent on the consumption of copper(II) oxide in the oxidizing section of the tube, and can extend to several weeks with moderate usage (provided that the copper(II) oxide is not spent, the oxidation–reduction sequence is quantitative and highly reproducible). An in-line water trap (magnesium perchlorate) is recommended, as even traces of water in the counting gas stream will shorten the life of the counting tube and high-voltage wire owing to corrosion. A carbon dioxide trap is also useful in eliminating this minor source of quenching when counting low levels of $^3H_2$.

This procedure described for radio-capillary gas chromatography was designed primarily for use with relatively volatile metabolites, such as monoterpenoids and sesquiterpenoids, to which unlabeled carriers are generally added in order to minimize evaporative losses during sample handling. However, the technique should be especially useful for products of lower vapor pressure (fatty acids, steroids, resins and carbohydrate and amino acid derivatives) for which carrier dilution is unnecessary, thus permitting greater flexibility in the choice of substrate-specific activity and the level of internal standard added.

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