

## A TEMPERATURE-PROGRAMMED PREPARATIVE-SCALE GAS CHROMATOGRAPH

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(Received October 16th, 1962)

During an investigation into the chemistry of essential citrus oils, a need developed for a large preparative-scale gas chromatograph so that sufficient quantities of some of the minor constituents could be separated for identification and study. A number of such chromatographs have been described<sup>1-4</sup> which required exceptional skill for construction or which had insufficient capacity. The temperature-programmed chromatograph, herein described, is of relatively simple design and was constructed of readily available materials. The resulting chromatograph handled 25 g of terpenes with resolution comparable to that of a 250-ft., 0.020-in. capillary column containing the same substrate.

### APPARATUS

The apparatus was constructed of two vertical round sheet metal ducts 8 in. and 6 in. in diameter respectively, approximately 11 ft. long connected so that hot air was circulated in a closed circuit up through the smaller duct containing electrical heaters and down through the larger duct containing the bundle of tubes. A photograph of the entire apparatus is presented in Fig. 1, a general schematic diagram is given in Fig. 2, the flash vaporizer is illustrated in Fig. 3, and the wiring diagram in Fig. 4.

### Column

Eight 1½-in. o. d. × 10-ft. columns were bundled as shown in Fig. 2 section A-A and fastened to the six air baffle plates making a rigid system. The ends of the stainless steel tubes were plugged, drilled, and tapped for ½-in. pipe to facilitate replacement of the packing. The eight tubes were connected in series using ¼-in. tubing and ½-in. pipe to ¼-in. tube fittings. Tube connections could be changed for column lengths of 20, 40, or 60 ft. The entire assembly was placed in the 8-in. × 11-ft. sheet metal duct, the latter being secured to the baffle plates with sheet metal screws. A removable insulated sheet metal cap was fitted to each end of the housing. Interconnecting ducts were secured to the upper and lower housing sides to receive the blower air at the top and exhaust it into the heater chamber at the bottom.

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\* One of the laboratories of the Southern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture. References to specific products of commercial manufacture are for illustration and do not constitute endorsement by the U.S. Department of Agriculture.

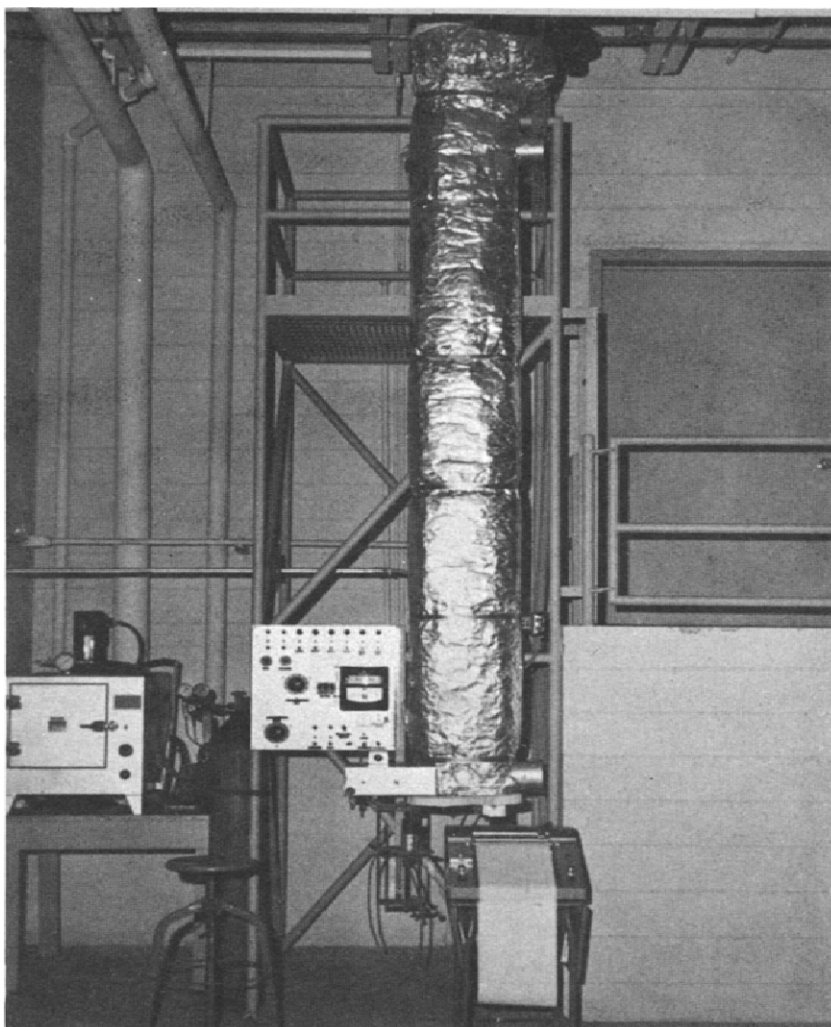


Fig. 1. Photograph of the temperature-programmed preparative-scale chromatograph.

### *Heaters*

The heaters were made from chromel heater coils cut into lengths to form three 1000-W and three 750-W elements. The elements were suspended from transite rings which were spaced with rods and the entire assembly inserted into the lower end of the 6-in. duct as shown in Fig. 2. The heaters were wired as shown in Fig. 4. The three 1000-W heaters and one 750-W heater were provided with on-off switches and fuses. One of the other 750-W heaters was controlled with a variable transformer and the other with a temperature indicating thermocouple controller and a variable transformer. Thermocouple leads were placed in a well extending 8-in. into the packing at the top of one of the  $1\frac{1}{2}$ -in. tubes, and in the air stream at the top, center, and bottom of the 8-in. duct. The temperatures were read on the indicating controller through a six-point switch. Control was from the thermocouple in the air at the top of the 8-in. duct. Air gates are shown at the top and bottom of the 6-in. duct and a damper toward the top of the duct. During operation, the gates were kept closed and the damper open. Rapid cooling of the column was achieved by opening the gates, closing the damper and allowing the blower to run.

Air was circulated through the apparatus by means of a pressure blower (Sutton

PB-45-A) rated at 300 c.f.m. against a static head of 3-in. of water. An anemometer inserted in the 6-in. duct showed an air velocity of about 3000 lineal feet per minute. Since the pressure blower was not designed especially for high-temperature work, a

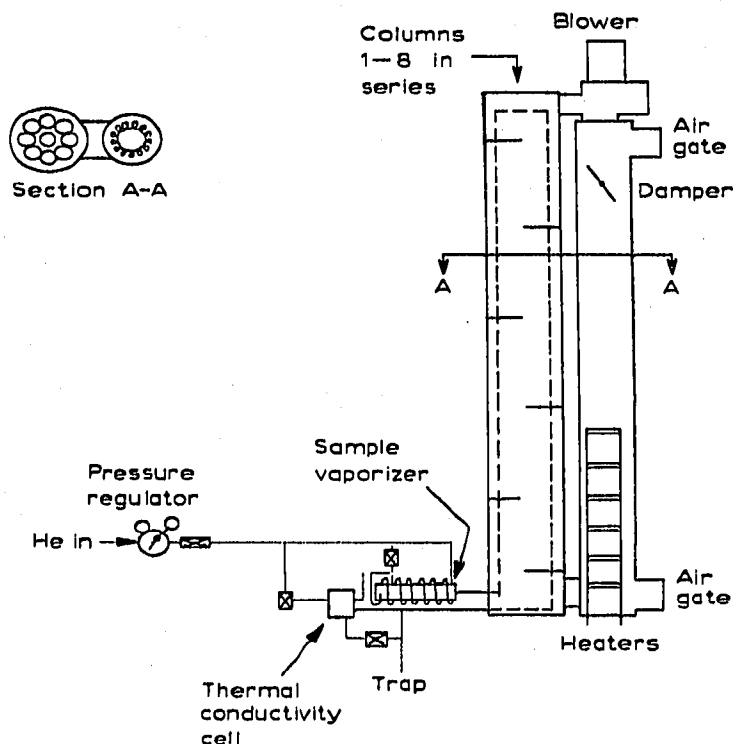


Fig. 2. Schematic flow diagram.

small air blower (40 c.f.m.) was installed and directed against the main blower motor to keep it from overheating.

The entire duct assembly was insulated with two 2-in. thicknesses of aluminum sided glass wool. The column housing caps which provide access to the column fittings were insulated with a 2-in. thickness of asbestos board.

#### *Thermal conductivity cell*

A full diffusion type hot-wire detector cell was constructed similar to the Gow-Mac TE-III. It was heated with a Chromolox 100-W Type A ring heater through a variable transformer as shown in Fig. 4. A thermocouple was inserted into the block and read on the indicating controller through the six-point selector switch. A portion of the eluting materials entered the cell through  $\frac{1}{8}$ -in. tubing after being split and reduced from  $\frac{1}{4}$  in. at the tee. The amount of material passing through the cell was controlled by a valve in the line just after the cell. The line from the valve was connected to the original  $\frac{1}{4}$ -in. tube leading to the trap. The reference gas was metered through the cell and exhausted. The entire unit was placed in a compartment to the side and bottom of the main column and packed with loose asbestos.

#### *Flash evaporator*

A flash evaporator was fabricated as shown in Fig. 3. A  $\frac{7}{16}$ -in. hole was drilled through a 1-in.  $\times$  6-in. stainless steel rod and the ends were tapped for  $\frac{1}{4}$ -in. pipe

fittings. A hole was drilled and tapped for a  $\frac{1}{4}$ -in. pipe fitting in the side,  $\frac{3}{8}$ -in. from one end. A  $\frac{1}{4}$ -in. tube to  $\frac{1}{4}$ -in. tube to  $\frac{1}{4}$ -in. pipe fitting was inserted into this tapped hole. One end of the cylinder was plugged and into the other end was placed

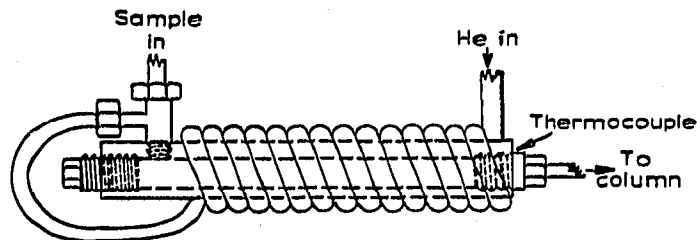


Fig. 3. Flash vaporizer.

a  $\frac{1}{4}$ -in. pipe to  $\frac{1}{4}$ -in. tube after the cavity was filled with Podbielniak stainless steel Heli-Pak 3013. The cylinder was wrapped with 22-gage asbestos insulated chromel wire and covered with a coil of  $\frac{1}{4}$ -in. copper tubing. A thermocouple was inserted into the cylinder end face and read on the indicating controller via the six-point selector switch. The entire unit was insulated with asbestos cloth tape, placed in the cell-flash evaporator compartment, and packed with loose asbestos.

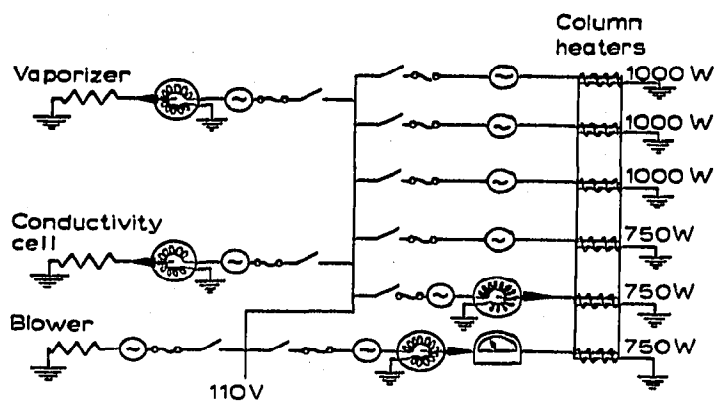


Fig. 4. Schematic wiring diagram.

A shut-off valve was placed on the injection port to which could be attached a pressurized cylinder containing the sample to be chromatogrammed.

#### *Temperature programmer*

The unit is capable of attaining  $160^{\circ}$  in 20 min, therefore, the column can be programmed at a maximum of  $8^{\circ}$  per minute. This can be accomplished by merely switching on the entire bank of heaters. For slower heating rates, the temperature indicator controller can be driven by any variable speed device. A surplus (gun fire control) ball-disc type variable speed drive, propelled by a 4 r.p.h. clock motor, was used successfully in the present apparatus. A temperature lag of  $15^{\circ}$  existed between the top and bottom of the column under these conditions; however, resolution was excellent.

#### PROCEDURE

The tubes were packed with 21 lb. of 30-60 mesh Chromosorb-P containing 30% Carbowax 20M and tapped with a hammer and punch until the material ceased to

settle. Nitrogen gas was passed through the column for all purposes except when materials were separated, at which time helium was used. Peak inversion<sup>5</sup> resulting from use of nitrogen as a carrier gas complicates the chromatogram of these materials at the temperatures required for separation. A pressure of 70 lb. p.s.i. was used resulting in a flow rate of 4 l/min.

Samples were introduced into the vaporizer under pressure using a 30 ml valved cylinder. In use, the sample cylinder was first evacuated, the sample was drawn in, and then pressurized to 4-5 times the column pressure. It was then connected to the valve on the sample vaporizer. Opening both valves permitted the entire sample to enter the vaporizer. A 25 ml sample could be injected and vaporized in 2-3 sec.

The block temperature was regulated at 150° and the flash evaporator at 180°. Both remained heated at all times. Because only a brief period was required to heat the column, it remained unheated, except when in use. For example, the column could be brought to equilibrium at 160° in 20 min.

#### APPLICATION

The preparative column was compared, for efficiency of separation, with a 250-ft. 0.020-in. capillary column. The parameters of this latter column were the same for obtaining the chromatograms shown in Figs. 5 and 7. The temperature was maintained at 100° with a helium flow of 15 ml/min resulting in a pressure of 36 lb. p.s.i. The 250-ft. column was coated with Carbowax 20M. An F & M 1609 flame ionization detector was used in conjunction with the F & M 500 gas chromatographic apparatus.

The preparatory column parameters were maintained the same for both chromatograms shown in Figs. 6 and 8. The column temperature was controlled at 150° with a helium pressure of 65 lb. p.s.i.

#### *Terpene hydrocarbons*

Fig. 5 shows the chromatographic separation of a 1- $\mu$ l sample of terpene hydrocarbons

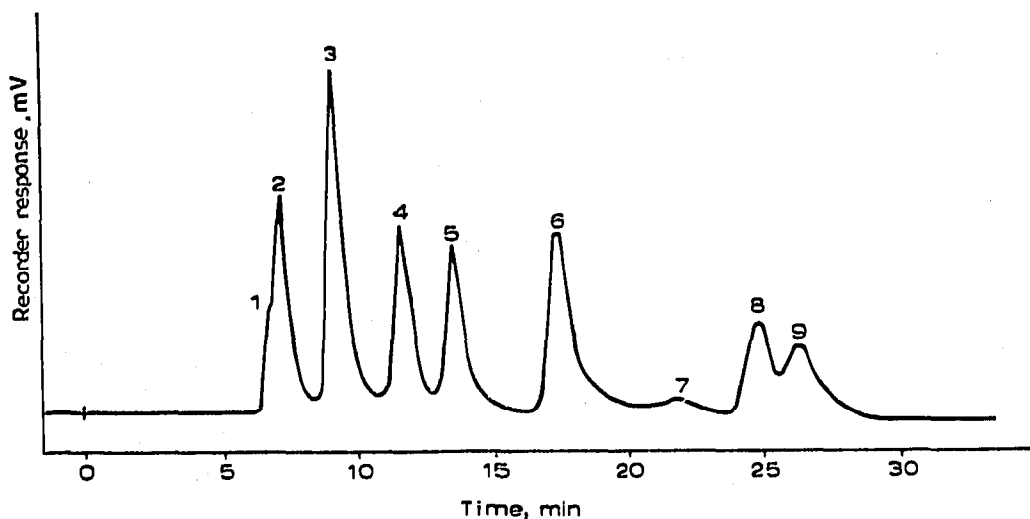


Fig. 5. Chromatogram of some hydrocarbon terpenes on a 250-ft. capillary column containing Carbowax 20 M, isothermal operation at 100°. Identified peaks: 1 = cyclofenchene; 2 =  $\alpha$ -pinene; 3 = camphene; 4 = sabinene; 5 = myrcene; 6 = *d*-limonene; 7 =  $\gamma$ -terpinene; 8 = *p*-cymene; 9 = terpinolene.

on a 250-ft. capillary column. The following peaks were obtained: cyclofenchene (1),  $\alpha$ -pinene (2), camphene (3), sabinene (4), myrcene (5),  $d$ -limonene (6),  $\gamma$ -terpinene (7),  $p$ -cymene (8), and terpinolene (9). The curve represents a 1- $\mu$ l sample which had been split. Fig. 6 shows the separation of 20 ml of this same terpene hydrocarbon mixture on the preparatory column. The separation of sabinene (4) and myrcene (5) is incomplete, however, by trapping the leading edge and the trailing edge separately, rather pure materials can be obtained.

#### Commercial terpinolene

A 1- $\mu$ l sample of Matheson Coleman and Bell terpinolene on the 250-ft. capillary column gave the chromatogram shown in Fig. 7. The peaks were identified by use of the preparatory column and confirmed by infrared spectroscopy as  $\alpha$ -pinene (A), camphene (B), myrcene (C), 1,4-cineole (D),  $d$ -limonene (E), 1,8-cineole (F),  $\gamma$ -terpinene (G),  $p$ -cymene (H), terpinolene (I), and isoterpinolene (J). Fig. 8 shows the separation of 20 ml of this same material on the preparatory column. In addition to the above peaks, the large column resolved  $\alpha$ -fenchene (A') and isoterpinolene (J). It should also be noted that  $d$ -limonene (E) and 1,8-cineole (F) have been more satisfactorily resolved.

Single peak material obtained from the preparatory column was rechromatographed on the 250-ft. capillary column and found to be chromatographically pure. Fig. 9 shows a temperature programmed separation of 10 ml of isoprene (b),  $n$ -pentane (c),  $n$ -hexane (d), and  $n$ -heptane (e) by means of the preparatory column. Nitrogen (a) was the injection gas. The parameters outlined above were maintained except the temperature was programmed at 2.5° per min from 60-120°.

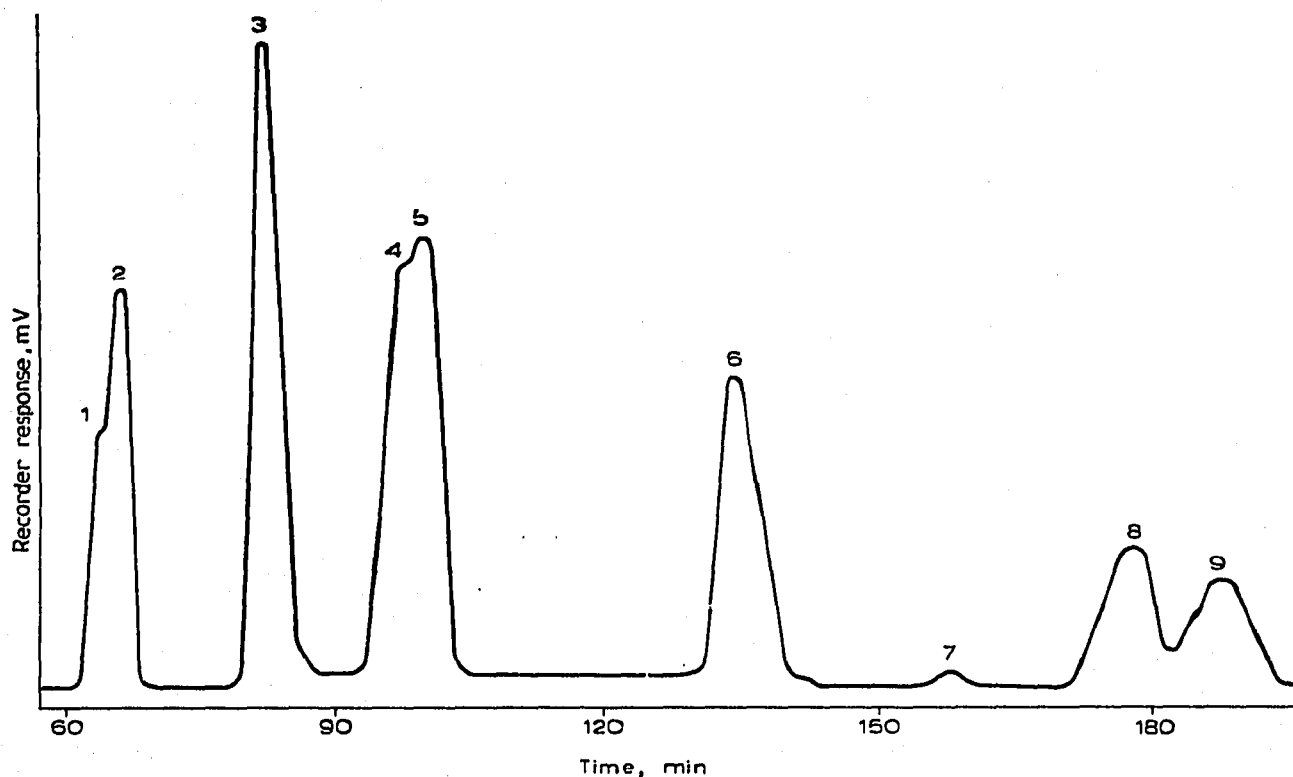


Fig. 6. Chromatogram of the hydrocarbon terpenes, shown in Fig. 5, on the preparatory column packed with 30% Carbowax 20M on 30-60 mesh Chromosorb P, isothermal operation at 150°.

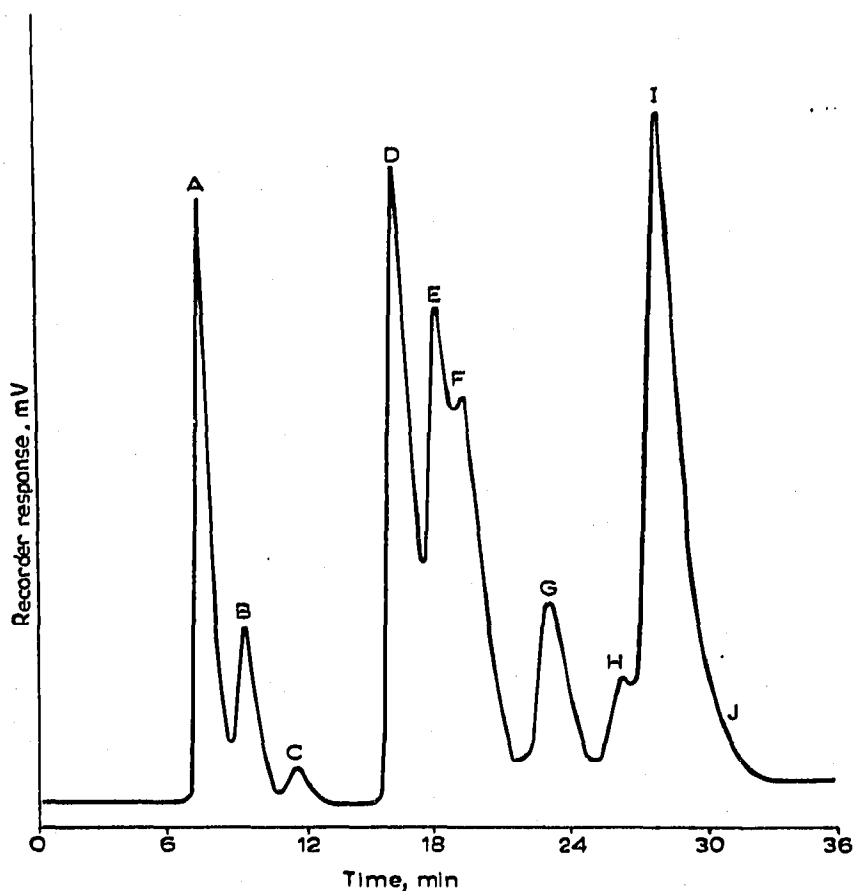


Fig. 7. Chromatogram of commercial terpinolene on a 250-ft. capillary column containing Carbowax 20M, isothermal operation at 100°. Identified peaks: A =  $\alpha$ -pinene; B = camphene; C = myrcene; D = 1,4-cineole; E = *d*-limonene; F = 1,8-cineole; G =  $\gamma$ -terpinene; H = *p*-cymene; I = terpinolene; J = isoterpinolene.

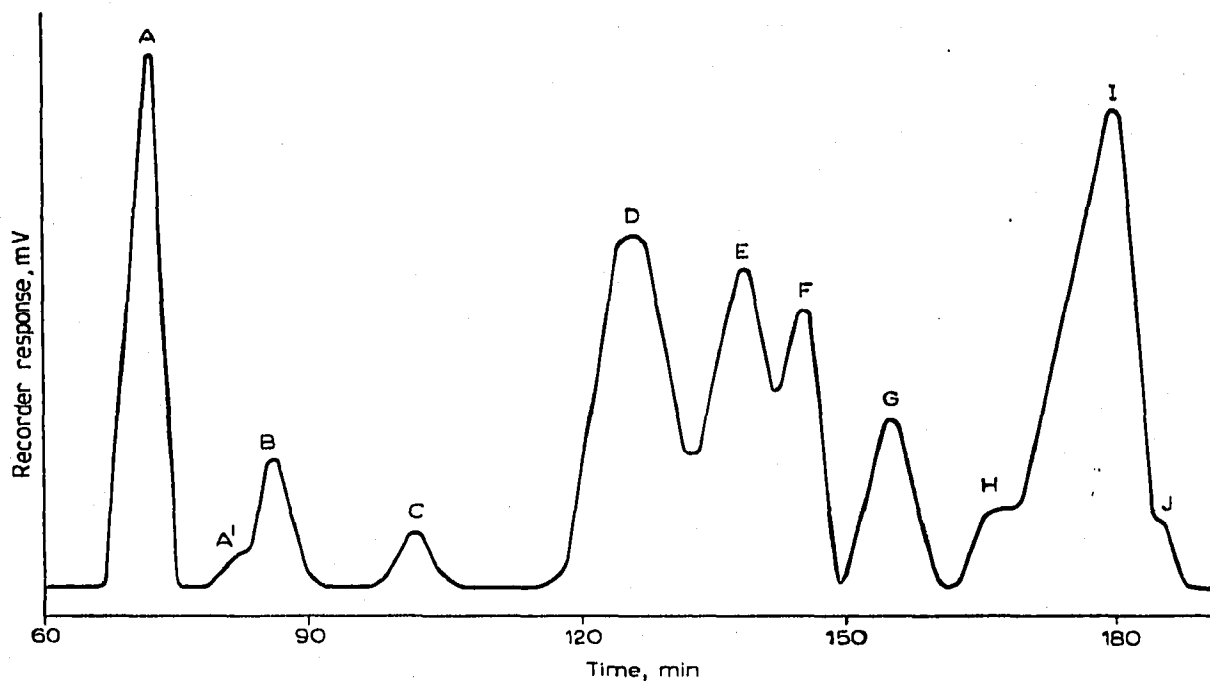


Fig. 8. Chromatogram of commercial terpinolene shown in Fig. 7 on the preparatory column at 150°, packed with 30% Carbowax 20M on 30-60 mesh Chromosorb P. In addition peak A' ( $\alpha$ -fenchene) appeared.

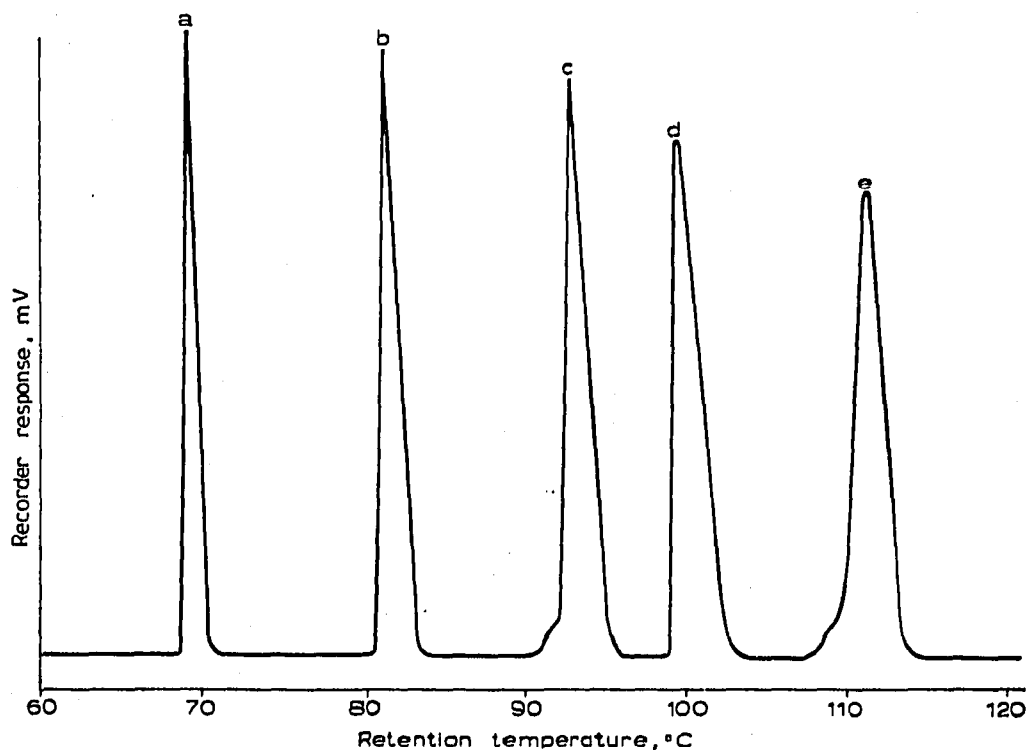


Fig. 9. Chromatogram of some hydrocarbons on the preparatory column temperature programmed at  $2.5^{\circ}/\text{min}$  from  $60\text{--}120^{\circ}$ . Peaks identified: a = nitrogen; b = isoprene; c = pentane; d = hexane; e = heptane.

#### ACKNOWLEDGEMENTS

The authors are indebted to W. B. BROGDEN for obtaining some of the chromatograms and in particular to C. E. KUNSMAN for its construction and many suggestions.

#### SUMMARY

An efficient temperature-programmed preparative-scale gas chromatography unit has been developed having  $1\frac{1}{2}$  in.  $\times$  10 ft. tubes connected in series. The unit is heated by thermostatically controlled hot air making possible temperature programming. The unit is capable of attaining a temperature of  $150^{\circ}$  in 15 minutes and can be cooled to  $50^{\circ}$  in the same time limit.

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