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GAS CHROMATOGRAPHIC SEPARATION OF MONOTERPENE HYDROCARBONS ON MODIFIED GRAPHITIZED CARBON BLACK

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SUMMARY

By coating the graphitized carbon black Carbopack C with a mixture of modifying agents, namely 1,2,3-tris-(2-cyanoethoxy)propane and N,N,N′,N′-tetrakis-(2-hydroxyethyl)ethylenediamine, the separation of the most common naturally occurring monoterpene hydrocarbons was achieved. Only 1,8- and 1,4-cineole are eluted as one peak and these can be separated on another column.

INTRODUCTION

Gas chromatography has been used extensively for the separation of terpene hydrocarbons but, although good results have been obtained, the complete separation of all naturally occurring terpene hydrocarbons has not yet been achieved. On the commonly used stationary phases, the separation of α-terpinene, α-phellandrene, limonene, β-phellandrene and γ-terpinene is the major obstacle. Additional overlapping peaks can be created by the presence of 1,8- and 1,4-cineole. Although these two compounds contain oxygen, the low polarity of the functional group coupled with the lack of two double bonds cause their retention times to be close to that of limonene.

Graphitized carbon black (GCB) has already been used for the separation of terpene hydrocarbons[1]. On the flat and non-specific surface of this chromatographic material, terpene hydrocarbons are separated on the basis of their geometrical structure and their probable orientation on the surface, irrespective of possible inductive and hyperconjugative effects of the substituents attached to an unsaturated carbon atom. For analytical purposes, however, unmodified GCB is unable to yield satisfactory separations of complex mixtures of terpenes. In addition, when an essential oil is injected, peak overlap between the monoterpene hydrocarbon fraction and oxygenated terpenes might occur.

GCB has been modified with 2% (w/w) of an acid phase, SP-1000 (Supelco, Bellefonte, Pa., U.S.A.), for the analysis of essential oils[2]. With regard to monoterpene hydrocarbons, this packing was found to be unsatisfactory for the separation of the mixtures 1,8-cineole–1,4-cineole–limonene–β-phellandrene and camphene–α-thujene.

The object of this paper is to show that the separation of the most common

naturally occurring monoterpene hydrocarbons in the presence of 1,8-cineole, 1,4-cineole and limonene epoxide can be accomplished by using the GCB Carbopack C treated with a mixture of the modifying agents 1,2,3-tris-(2-cyanoethoxy)propane (TCEP) and N,N,N′,N′-tetrakis-(2-hydroxyethyl)ethylenediamine (THEED). Under the conditions used, only 1,8-cineole cannot be separated from 1,4-cineole. However, another column packing can be used for the complete separation of this pair in the presence of terpene hydrocarbons.

EXPERIMENTAL

Carbopack C (80–100 mesh) was supplied by Supelco and was ground to 100–120 mesh.

Column packings were prepared by dissolving weighed amounts of TCEP and THEED in methanol or acetone and adding the solution to a known weight of GCB in a flat dish. The packings were dried slowly at room temperature (20–22°) without stirring, as this operation would crush the GCB particles. The dried materials were re-sieved accurately so as to maintain the proper mesh range.

Coiled glass columns of I.D. 1.8 mm were packed with this material with the aid of a vibrator. The packing operation is critical and, in order to obtain high-efficiency columns, it is recommended that the following procedure should be followed closely. The packing material is added to the column by means of a funnel and the column is vibrated gently and continuously in a uniform manner without shocks, starting from the bottom and slowly moving up to the top. The column must always be rotated in the same direction. Vibration causes some re-adjustment of the GCB particles, which are more closely packed the more uniform and regular this operation is carried out along the full length of the column. Vibration is repeated several times and the packing can be terminated when a further vibration set from the bottom to the top affects the level of the carbon inside the column by less than 0.2 mm. When a column is correctly packed, the amount of Carbopack C should be about 0.89 g per millilitre of column volume.

After packing, the columns were conditioned overnight at 80°. A Carlo Erba Model GI gas chromatograph was used, connected to a Leed and Northrup Speedomax recorder operating with a 1-mV full-scale response. At the maximum sensitivity of the amplifier system (1 × 1), about 1.5 pA gave a full-scale response on the recorder. Extra-pure hydrogen was used as the carrier gas. When gases of ordinary purity were used, slight variations in the separation factors of terpenes were noted.

RESULTS AND DISCUSSION

Fig. 1a is a graph of retention times of terpenes relative to that of limonene at 80° *versus* the percentage of TCEP added to the carbon surface. According to Klouwen and Ter Heide[3], a highly polar liquid phase, such as TCEP, was found to have the highest selectivity towards terpenes. These isomeric compounds may show large differences in polarizability, arising from the different effects of the polarizable substituents on the neighbouring double bonds. In this respect, the separation power of TCEP is based upon differences in dipole-induced dipole-type interactions.

As shown elsewhere[4,5], capacity ratio data for a suitable eluate as a modifying

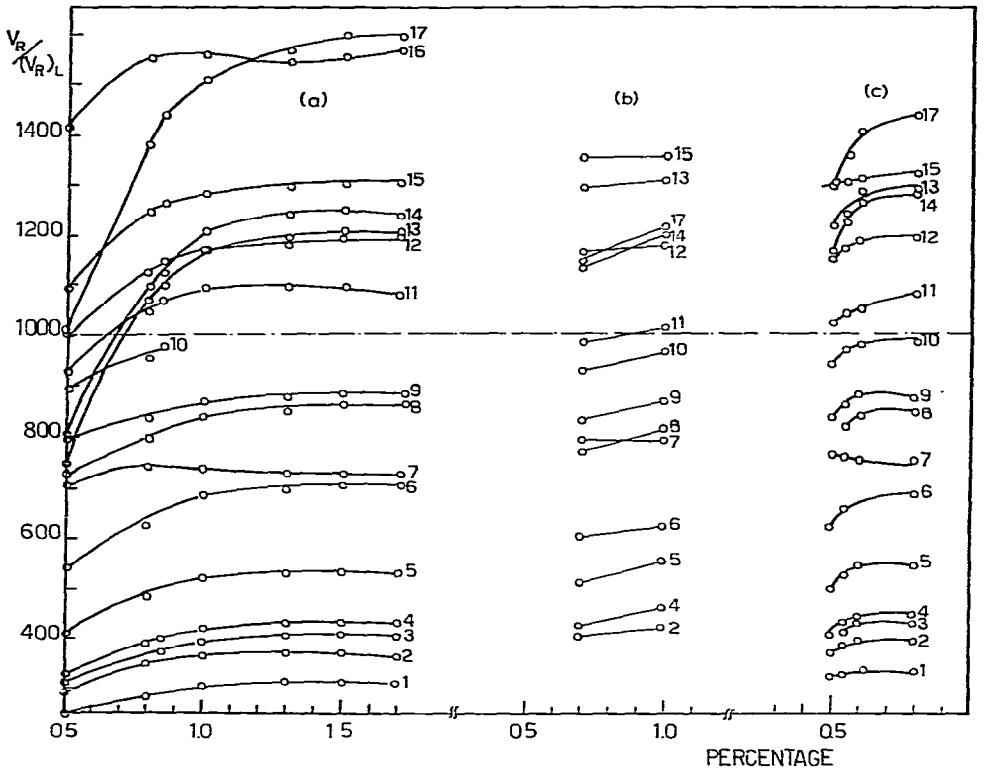

Fig. 1. Plots of corrected retention times relative to limonene for some terpenes at 80° *versus* the amount of (a) TCEP, (b) THEED and (c) TCEP + 0.5% THEED added to Carbopack C. 1, Tricyclene; 2, α-pinene; 3, α-thujene; 4, camphene; 5, β-pinene; 6, sabinene; 7, Δ^3-carene; 8, myrcene; 9, α-phellandrene; 10, α-terpinene; 11, β-phellandrene; 12, 1,8-cineole; 13, 1,4-cineole; 14, γ-terpinene; 15, terpinolene; 16, *p*-cymene; 17, *cis*-ocimene.

agent is added to a homogeneous adsorbing medium can be used to estimate the monolayer capacity. In this work, a complete monolayer of TCEP molecules could be estimated to occur at a coverage of about 0.5% (w/w). Therefore, within the range of TCEP percentages taken into consideration, retention of eluates is due to the combined effects of sorption into the multi-molecular film and adsorption on its outer layer. In this instance, the adsorbing surface of GCB plays an indirect role in the chromatographic process, as the selectivity characteristics of the adsorbed film are strictly dependent on the mode of adsorption of TCEP molecules[6].

As can be seen, on increasing the concentration of TCEP from 0.5 to 1%, *i.e.*, from one to two molecule thick adsorbed layers of TCEP, there are sharp variations in the relative retention times. This effect indicates that the chromatographic process changes rapidly from adsorption on the monolayer to some kind of solution into the bimolecular film of TCEP. At TCEP concentrations higher than 1%, changes in the relative retention times become smaller, as the effect of adsorption is increasingly overwhelmed by the effect of solution in TCEP.

A 0.5% TCEP column exhibits good selectivity characteristics. On the other hand, under these conditions terpenes appear to be retained too strongly, making the

analysis time too long. At TCEP concentrations above 0.5%, α-terpinene tends to be eluted rapidly with limonene and the separation of 1,8-cineole from γ-terpinene is poor.

Fig. 1b is a graph of retention times relative to that of limonene at 80° *versus* the percentage of THEED added to the carbon surface. It can be seen that the presence of THEED molecules on the carbon surface generally produces an effect similar to that of TCEP molecules. Contrary to TCEP, THEED is able to retard 1,8-cineole considerably with respect to γ-terpinene. This effect can be explained by considering that the oxygen atom in 1,8-cineole can establish a weak hydrogen bond with the hydroxyl groups of the modifying agent. This effect does not occur with 1,4-cineole. The reason for this anomalous behaviour is unclear, and only geometrical effects upon adsorption on the THEED layer opposing the formation of hydrogen bonds can be suggested.

Another effect of adding THEED to the carbon surface is that, at equal surface concentrations, THEED-modified GCB shows a more favourable separation factor than TCEP-modified GCB for the pair α-terpinene–limonene.

Therefore, with a view to obtaining the separation of terpenes, we used GCB modified with a mixture of TCEP and THEED. Fig. 1c is a graph of relative retention times *versus* the percentages of TCEP added to carbon modified with a constant concentration of 0.5% of THEED. With the exception of 1,8-cineole, it can be seen that the order of elution on TCEP-modified GCB is not altered by the addition of THEED.

Although 1,4-cineole cannot be separated from 1,8-cineole, GCB modified with

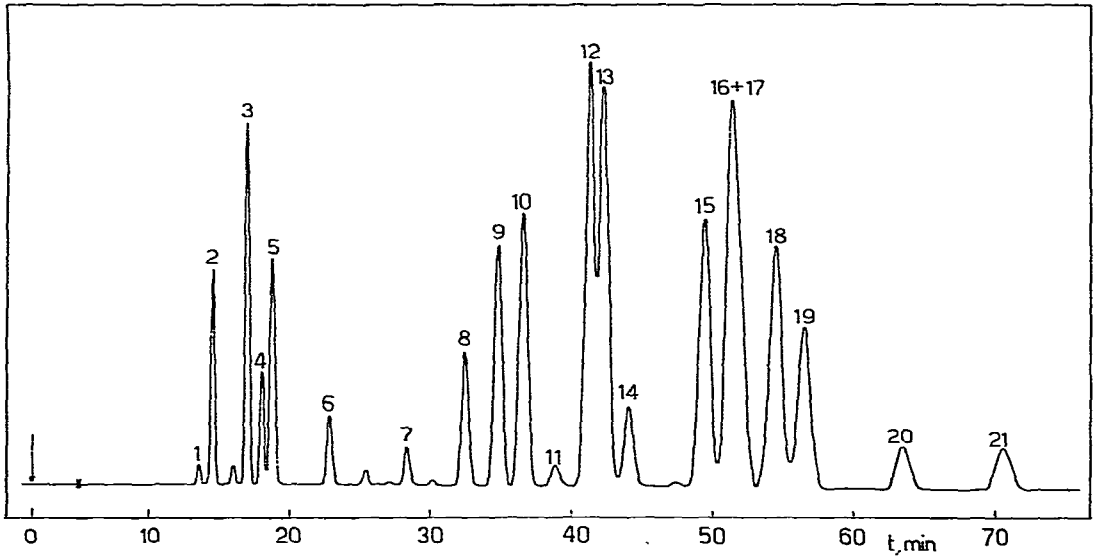

Fig. 2. Chromatogram of a complex terpene mixture at 80° on a 5 m × 1.8 mm I.D. glass column containing Carbopack C (100–120 mesh) modified with 0.5% of THEED and 0.55% of TCEP. Pressure drop, 5 kg/cm²; linear carrier gas velocity, 8.7 cm/sec. 1, Santene; 2, tricyclene; 3, α-pinene; 4, α-thujene; 5, camphene; 6, β-pinene; 7, sabinene; 8, Δ^3-carene; 9, myrcene; 10, α-phellandrene; 11, limonene epoxide; 12, α-terpinene; 13, limonene; 14, β-phellandrene; 15, γ-terpinene; 16, 1,4-cineole; 17, 1,8-cineole; 18, *p*-cymene; 19, terpinolene; 20, *cis*-ocimene; 21, *trans*-ocimene. Carrier gas: hydrogen.

0.5% of TCEP plus 0.5% of THEED appears to yield the optimal selectivity characteristics for the chromatographic analysis of a complex mixture of terpenes. With lower percentages of TCEP *p*-cymene cannot be separated from terpinolene nor 1,4-cineole from γ-terpinene. On the other hand, with higher percentages of TCEP α-terpinene and limonene tend to be eluted together and so do 1,8-cineole and terpinolene.

Even under optimal experimental conditions some pairs of terpenes have low separation factors. However, a 5-m column packed with 100–120-mesh GCB particles, having an efficiency of about 21,000 plates[7], was used to achieve the separation of terpenes. The chromatogram obtained at 80° is shown in Fig. 2. Hydrogen was used as the carrier gas[8,9] in order to reduce the analysis time. Peak 11 is indicated as limonene epoxide. We have no standard for this compound, but its presence could be detected by gas chromatography–mass spectrometry, as it is contained as impurity in a terpinolene sample used to prepare the artificial mixture of terpenes. Fig. 3 shows the relative mass spectrum together with the structural formula of limonene epoxide. From the examination of the mass fragments of the unknown compound, it could be concluded that peak 11 corresponds to the elution of limonene epoxide.

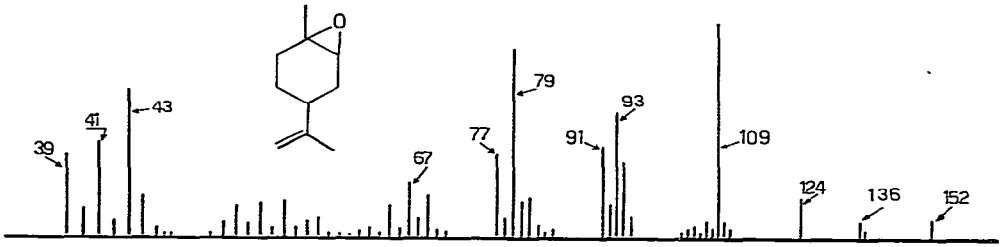

Fig. 3. Electron-impact mass spectrum relative to peak 11 (Fig. 2) and the structural formula of limonene epoxide.

1,4- and 1,8-cineole were eluted as one peak, but their baseline separation without overlapping of the peaks of other terpenes could be obtained at 72° by using a 3-m column packed with 0.7% THEED-modified Carbopack C (100–120 mesh).

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