

CHROM. 4440

The separation of alkanes on Sephadex LH-20 and its application to a rock extract

The separation of alkanes by preferential sorption into inorganic molecular sieves (Union Carbide 5A and 7A) has been shown to be an important technique in the analysis of alkanes of geological interest^{1,2}. Sephadex LH-20 (Pharmacia), a methylated dextran gel, which behaves as a molecular sieve, has also been used to separate alkane mixtures when used in column chromatography^{3,4}. This paper describes more fully the potential of LH-20 in separating cyclic alkanes from acyclic alkanes of similar molecular weight.

Method and results

The starting materials were a mixture (A) prepared from alkanes of various structures (Table I) and a naturally occurring mixture (B) of branched and cyclic alkanes isolated by usual methods⁵ from an organic extract of Green River Shale.

TABLE I

COMPOSITION OF MIXTURE A

The alkanes were present in approximately equal amounts.

Group	Carbon numbers and names
<i>n</i> -Alkanes	12, 14, 16, 18, 22, 24, 26, 28
Isoalkanes and anti-isoalkanes	13, 15, 16, 18, 24
Acyclic isoprenoid alkanes	15-farnesane, 19-pristane, 30-squalane
Cyclohexyl alkanes	16, 18, 19, 21
Tetracyclic alkanes	19-androstane, 27-cholestane

A slurry of LH-20 (approx. 90 g) was made with an acetone-chloroform mixture (1:1) and sedimented into a silicone-treated glass chromatography column (200 cm × 1.5 cm). The column was equilibrated by elution with solvent mixture (200 ml) and allowed to stand for 24 h. The gel then occupied 150 cm (270 ml) of the column.

Mixture A (9.7 mg in 0.5 ml petroleum ether b.p. 40–60°) was pipetted onto the top of the column of gel and allowed to be absorbed. Solvent-washed cotton wool was inserted onto the gel surface and the solvent mixture added. Eluant (100 ml) was run off from the bottom of the column and then fractions of 1.1 ml were collected (flow rate 40 ml/h). The alkane mixture occurred in fractions 14 to 63. Each of these fractions was evaporated at 40° under a nitrogen stream and redissolved in benzene (20 μl) containing internal standards of cyclohexylnonane (1 μl/ml) and dotriacontane (2 mg/ml). Analyses were carried out by GLC on a 10 ft. × $\frac{1}{8}$ in. O.D. column of 3% OV-1 on 100–120 mesh Gas-Chrom Q (Applied Science), programmed at 10°/min from 70° to 290°.

The amounts of each component in each fraction were calculated as percentages of amounts in the original mixture (*e.g.* Fig. 1) and the number of the fraction containing the maximum concentration of an alkane was plotted against the carbon number of that alkane (Fig. 2), the fraction numbers being on a logarithmic scale. There was no

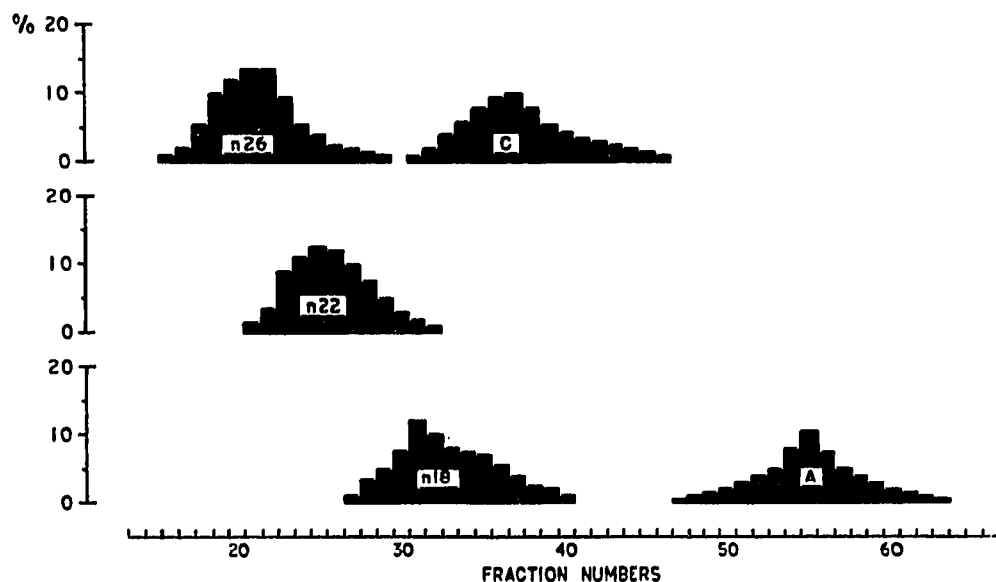


Fig. 1. Percentage of original sample present in fractions. *n*-26 = *n*-hexacosane; *n*-22 = *n*-docosane; *n*-18 = *n*-octadecane; C = cholestane; and A = androstane.

separation of normal from monomethyl alkanes and these alkanes (in Fig. 2) lie on a straight line (*n*). The cyclohexyl alkanes also produced a straight-line plot (*c*) displaced to higher fraction numbers. Between these two lines are the acyclic isoprenoid alkanes: squalane, pristane and farnesane. The tetracyclic alkanes cholestane and androstane were strongly retarded in elution from the column. It is seen, from the data presented, that the molecular sieving effect which separated *n*-hexacosane from *n*-octadecane is subordinate to another factor, possibly a solvent-solute interaction, which allows separation of *n*-octadecane from androstane.

The sample of Green River Shale branched and cyclic alkanes (28 mg in 0.25 ml

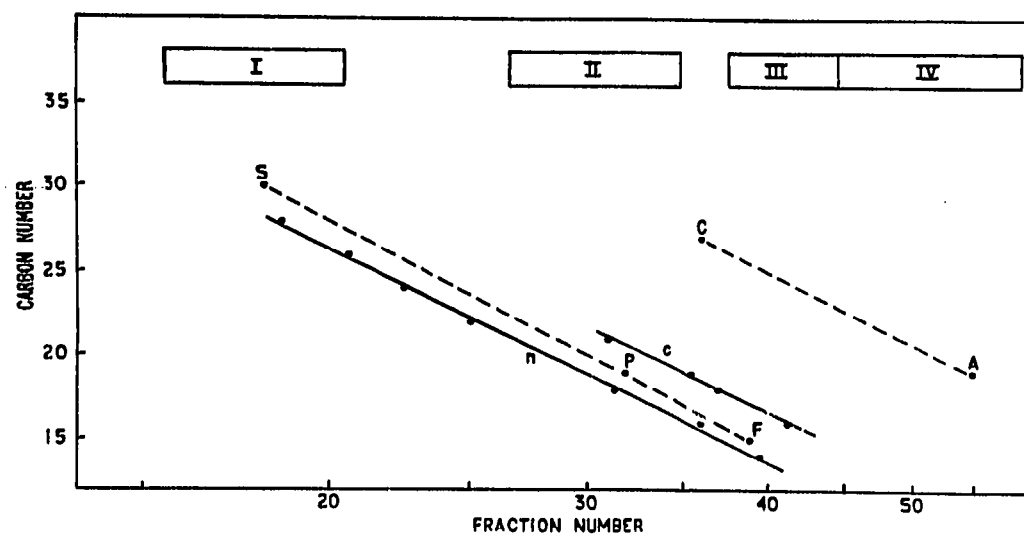


Fig. 2. Position of elution of the *n*-alkane series (*n*), the cyclohexyl alkane series (*c*), squalane (S), pristane (P), farnesane (F), cholestane (C) and androstane (A) and the fractions I-IV plotted against carbon number.

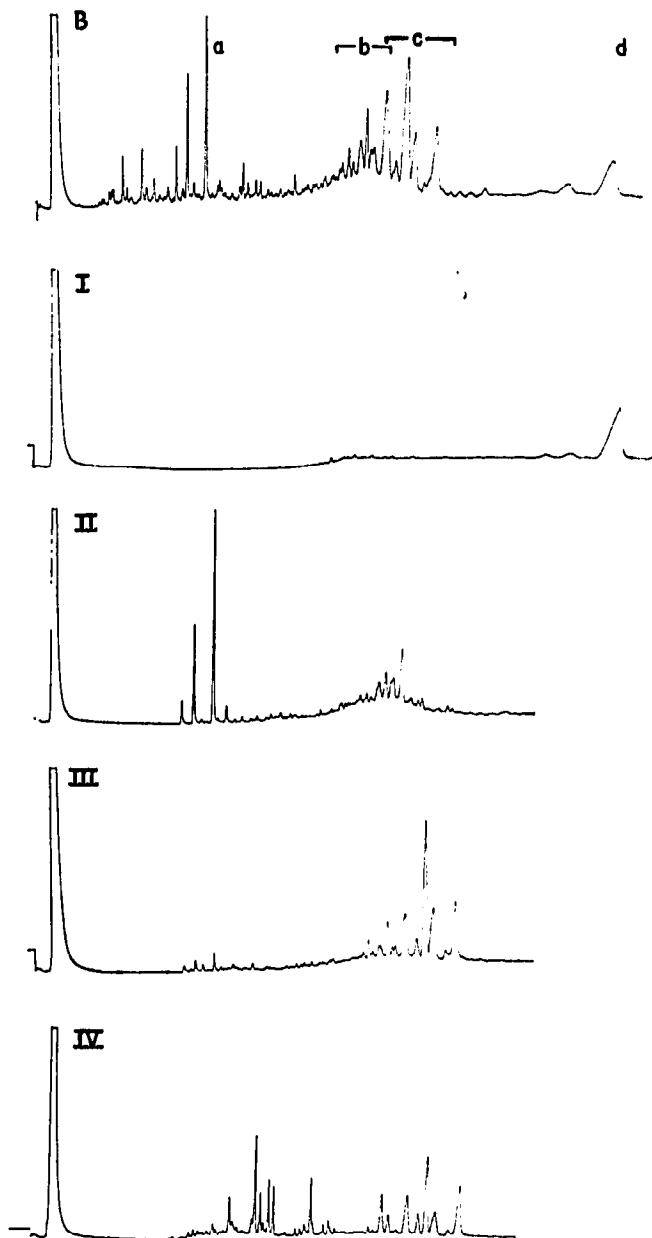


Fig. 3. GLC traces of the Green River Shale branched and cyclic alkanes fraction (mixture B) containing phytane (a), steranes (b), triterpanes (c) and perhydro- β -carotane (d) and the subsequent fractions I-IV.

petroleum ether) was passed through the column and appeared in fractions 16 to 60. After concentration and re-solution in benzene (without internal standards) analysis by GLC was carried out as before. Inspection of the gas chromatographs of each fraction allowed collection into larger fractions as follows:

- I (16-20), containing only perhydro- β -carotane (d),
- II (27-34), containing phytane (a), pristane, and steranes (b),
- III (38-44), containing triterpanes (c),
- IV (45-59)

Information on the identity of the alkanes was derived from the publications of previous workers⁵⁻⁷. The gas chromatograms of the original alkane mixture, and of the subsequent fractions I-IV, are shown in Fig. 3. Again it was seen that polycyclics were retarded relative to linear alkanes of the same carbon number, so that triterpanes were eluted after phytane. The separation also allowed the concentration of a group of alkanes in fraction IV with carbon numbers between 18 and 24 which had been minor constituents of the original mixture. From their behaviour on the column, it is believed that they have tri- and tetracyclic structures.

This method can be used advantageously for preliminary separation and concentration of alkanes prior to preparative GLC and GLC-mass spectrometry, or for the separation of components which have similar retention times on GLC. There appears to be no chemical breakdown as happens with methods involving refluxing but the large amounts of solvents evaporated in the processing may cause loss of volatile alkanes of low molecular weight.

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