

CHROMATOGRAPHIC SEPARATION AND CHARACTERIZATION OF HOPANES
IN ATHABASCA BITUMEN

T.C.S. Ruo, M.L. Selucky, Y. Chu and O.P. Strausz

Hydrocarbon Research Centre
Department of Chemistry, University of Alberta
Edmonton, Alberta, Canada, T6G 2G2

INTRODUCTION

One of the more difficult tasks in the analysis of heavy crudes such as shale or oil sand bitumens is the detailed analysis of their saturated hydrocarbon fraction. Although this fraction is easily separated from the other constituents of the bitumen in two or even in one separation step, it represents a complex mixture of hydrocarbons which may, and in the case of heavy crudes often does, contain several classes of compounds ranging from simple straight chain alkanes, to various branched alkanes (and possibly the corresponding olefins which are eluted together with the saturates), to monocyclic, dicyclic and up to pentacyclic compounds. Separation of this complex mixture into the individual classes is a formidable task which, in the case of heavy crudes, is further complicated by the fact that the constituents of this fraction range from relatively low molecular weight compounds up to C_{40} and even higher hydrocarbons. Also, as the number of rings increases, the corresponding number of possible stereoisomers becomes very large. Thus, for example, GALLEGOS¹ showed that three hydrocarbons of molecular formula $C_{30}H_{52}$ were present in the Green River shale oil: two had identical mass spectra, while the third differed only by the absence of a small M-43 peak in its mass spectrum. All three could be separated by GC. The slight differences in the mass spectra and the presence of common base ions (e.g. the majority of tetra- and pentacyclic terpanes have a major m/e 191 fragment in their mass spectrum) make discriminations based on GC/MS extremely difficult.

In heavy bitumens such as Athabasca or Cold Lake materials, and materials from the neighbouring areas, the major proportion of the hydrocarbons ranges between C_{16} and C_{30} . In this range GC/MS cannot easily furnish the entire structural data necessary for unambiguous identification of individual compounds, but other instrumental methods such as X-ray diffractometry or ^{13}C n.m.r. can provide the required information. These methods however require preparative isolation of the compound of interest in quantities amounting to at least 5-15 mg. WSZOLEK et al. therefore studied preparative isolation of the steranes and hopanes from the Green River shale oil².

Recent geochemical work has shown that many constituents of the saturated hydrocarbon fraction are hydrocarbons derived from naturally-occurring plant (e.g. terpenes and sterols) and possibly animal (viz. e.g. the recent identification of cholic acid by SEIFERT³) materials. Geochemists have pointed out repeatedly that such compounds, termed 'geochemical markers', might provide an insight into the genesis of the deposit and help to answer such questions as the relative maturity of the crude, and whether biodegradation has taken place. With regard to the latter, for example, it has been shown⁴ that certain bacteria are capable of attacking and thus removing n-alkanes, branched alkanes and even monoaromatics with an alkane side-chain and since these compounds are absent in the Athabasca bitumen⁵ it was concluded that complete biodegradation has taken place. However not too much importance should be vested in the presence or absence of other so-called geochemical markers since many terpanes and steranes are apparently always present in oils, irrespective of their origin and maturity.

In order to exploit the potential value of such classes of hydrocarbons for oil characterization studies, further refinements of existing analytical procedures were deemed necessary in order to allow routine (i.e. at least relatively fast and accurate) analyses and unambiguous identifications of the individual members of such classes of hydrocarbons. In view of the above-mentioned broad distribution of saturated hydrocarbons in the Alberta oil sand bitumens, we decided to first concentrate on the separation and determination of the hopanes and steranes using a combined GC/MS, HPLC and GPC approach.

RESULTS AND DISCUSSION

A well established but tedious and lengthy⁶ procedure for the isolation of penta-cyclic terpanes consists of the removal of possible olefinic contaminants on Ag⁺/silica, n-alkanes on 5A molecular sieve, of lower cyclic and branched open chain hydrocarbons by adduction with urea, and of higher cyclic compounds by adduction with thiourea. The hopanes remain in the thiourea non-adduct, while the steranes are distributed between the thiourea adduct and non-adduct. In previous work we have shown⁵ that, from the total 'saturates' of Athabasca oil, 1.53% were retarded on Ag⁺ loaded silica, 4.9% represented the thiourea adduct and 87.5% were isolated as the thiourea non-adduct after repeated adduction, for a total recovery of 94%. Thus the thiourea non-adduct represents the major portion of the saturate fraction. Due to the large spread in molecular weight of the saturates, it seemed obvious that some size separation should precede any other separation steps. A detailed study of the elution of saturates from a classical alumina column in which the eluate was collected in 10 separate fractions, each of which was checked by GC and HPLC (Figures 1 and 2) showed that the hopanes were preferentially eluted in fraction #7 and only small amounts were present in fraction #6. Since no n-alkanes are present in the Athabasca bitumen, fractions #5, 6 and 7 were treated with thiourea and the non-adduct was subjected to GC/MS scanning.

This scanning, based on the comparison of the intensities of the fragment ion m/e 191 and of the pair molecular ion plus an ion representing the side chain-containing fragment of the particular compound, suggested the presence of hopanes⁷ but the results were hard to interpret due to incomplete separation and the presence of steranes and other material in the same range of retention times, c.f. Figure 3. In order to improve the separation, each of the fractions (#5, 6 and 7) were re-separated by GPC on 60, 100, 200 and 500 Å Styragel. Examples of the GPC chromatograms and the cut points of fractions #5, 6 and 7 are shown in Figures 4a, b and c. The gas chromatograms of these individual fractions are shown in Figures 5, 6 and 7 where it is seen that the hopanes are preferentially concentrated in one of the fractions (GPC fraction #8 from alumina fraction #7). Thus the lower molecular weight constituents were nearly completely removed, leaving a mixture of hopanes together with traces of other components as will be shown below, reasonably well separable on a conventional packed GC column (Dexsil-300). This finding was important for two reasons. First, it allowed us to obtain good mass chromatograms and mass spectra of the individual hopanes, the fragmentation patterns of which were in agreement with published data⁷ on the 17 α (H), 21 β (H)-, C₂₇, C₂₉-C₃₅ hopane series, and second, it suggested the possibility of a relatively easy preparative collection of enough material to run ¹³C-n.m.r. and high resolution mass spectra of the individual compounds. For the preparative separation, a 1/4" by 8' column packed with 3% Dexsil-300 on Chromosorb W-AW was used with the thermal conductivity detector connected to the column outlet prior to the collection traps. The chromatogram is shown in Figure 8. Unfortunately the trapping train was only crudely set up and the collection efficiency was low. Comparison of the chromatograms of the isolated fractions with that of original hopane fraction, Figure 9, shows that the purity of the isolated C₂₇, C₂₉ and C₃₀ hopanes was better than 90%. Moreover, these hopanes are the predominant representatives of the hopane series in Athabasca bitumen, in which the total content of hopanes is about 0.3-0.6%. High resolution mass spectra of the C₂₉ and C₃₀ compounds, Figure 10, have exact masses of 398.3911 (calc. 398.3912) and 412.40690 (calc. 412.40644) respectively, thus confirming the molecular formulae C₂₉H₅₀ and C₃₀H₅₂. Comparison of the ¹³C n.m.r. spectra with that of C₃₀ hopane⁸, Figure 11, shows that the hopanes in Athabasca bitumen are indeed the 17 α (H), 21 β (H)-isomers.

Figure 12 illustrates the procedure adopted for GC-MS scanning of one of the GPC fractions containing C₂₉, C₃₀ and C₃₁ hopanes. The respective molecular ions are m/e 191 and the fragments containing the side chain are, respectively, m/e 177, 191 and 205. We have also detected and preparatively isolated a C₂₈ compound which gave fragment ions and a molecular ion in accordance with the other homologues of the now unambiguously identified hopane series, but the retention time however does not conform to the retention times of this series. This suggests that the compound is not a C₂₈ hopane and the fact that it could not be crystallized suggests that the isolated product might in fact be a mixture.

A gas chromatogram of the hopane concentrate fraction on a SCOT column (N = 28,000), Figure 13, reveals the presence of a number of trace and minor constituents which, however, could hardly affect the purity of the isolated fractions for the purpose of ^{13}C n.m.r. spectra or X-ray crystallography. Figure 13 clearly shows that the separation of the diastereoisomers at C-22 of C_{32} to C_{35} hopanes is complete, while the analogous separation of the C_{31} homologue would require efficiencies in the range of N = 80,000. It is also noteworthy that the ratios of the pairs of diastereoisomers change slightly along the series. From the limited data available however, the potential significance of these differences cannot be assessed. Figure 14 shows the same separation on a WCOT column (N = 80,000). The separation of C-22 diastereoisomers is nearly complete ($t_R \sim 47$ min.). However, the C_{28} compound gives a single peak even at this high resolution. Apparently, the material isolated by preparative GC is appreciably contaminated by neighboring components.

Similarly, cross-scanning for steranes has been done by molecular ion and fragment ions m/e 217 and 231, and fragment ions m/e 149 and 151. The ion m/e 217 is a base fragment of steranes and m/e 231 is the base ion of sterane compounds bearing a skeletal methyl group. The ratio of m/e 149 and 151 ions allows discrimination between the 5α and 5β stereoisomers of the sterane series. We have identified steranes ranging from C_{21} to C_{29} and work is still in progress. Figure 15 is an example of the GC/MS scanning of one of the GPC fractions in which the steranes are concentrated. Thus, in this fraction the following could be identified: a tetracyclic compound (terpane? m/e 191) $\text{C}_{19}\text{H}_{32}$ (mass 260), a tricyclic compound (m/e 191) $\text{C}_{20}\text{H}_{36}$ (mass 276), a tetracyclic hydrocarbon $\text{C}_{20}\text{H}_{34}$ (mass 274), mixed peaks consisting of C_{21} methylsterane and C_{20} sterane, C_{22} methylsterane and sterane, C_{23} methylsterane and sterane, a $\text{C}_{24}\text{H}_{42}$ tetracyclic terpane (m/e 191), a $\text{C}_{27}\text{H}_{48}$ tetracyclic terpane (mass 372) and a C_{27} hopane. Also, minor amounts of C_{29} , C_{30} and C_{31} hopanes were present.

It was important that a fairly good separation be achieved on a conventional GC column. The GC/MS equipment used for the measurements (MS-12 mass spectrometer interfaced to a Nova DS-50 data system) did not permit the use of WCOT columns without modifications to the sample introduction system in the MS. On the other hand the advantage of this system is the possibility of storing the large number of spectra (500 to 600 scans) and recalling any mass chromatogram or their combination off-line. The separation achieved on the classical liquid chromatography column and the resulting concentration of the hopanes in two of the fractions indicated that the length of time involved in the procedure could be substantially shortened if the efficiency of the column were increased. The hopanes should be easy to isolate under such circumstances since they are the highest molecular weight representatives of the compound spectrum of the saturate fraction. This expectation was confirmed by experiment. The total saturate fraction (about 1 g) was injected on a 2 cm i.d. x 4' column packed with 37-63 μm particle size alumina and eluted with n-pentane using a Chromatronix pump at a flow rate of 18 ml/min. to give the chromatogram shown in Figure 16. Cutting into subfractions as shown in Figure 16 afforded two main fractions, the gas chromatograms of which are compared in Figure 17. These chromatograms show that upon proper cutting, the hopanes can be very well separated from the total saturates and be isolated in one preparative step in only 35 minutes. Thus, an increase in the number of theoretical plates to only about 400/m yielded a reasonably pure hopane fraction.

The use of high performance alumina columns (particle size 10 or 5 μm) would further improve the separation. However, the low loadability of such columns, the high back pressures generated when the column is used at optimum conditions and the high cost of such columns indicate that a compromise solution, e.g. the use of 18-32 μm particle size in a preparative column, should give the best results. An additional problem related with the use of alumina columns which should not be overlooked is the strong influence of its water content on k-values. ENGELHARDT⁹ described a simple assembly which is capable of adjusting the water content and thus k-values to a selected, constant value. We have, however, preferred to work with a non-linear isotherm as illustrated in the chromatogram in Figure 16 using fully activated (400°C) alumina and freshly dried eluent (n-pentane).

This procedure for the separation and identification of the hopane constituents in the saturated hydrocarbon fraction is relatively simple and fast, and moreover the

capillary GC results indicate that the component distribution can be accurately quantified. A large number of comparative analyses can be performed routinely, possible variations in the distribution and ratios of diastereoisomers can be accurately determined and therefore the importance of these variables with regard to the geochemical history of the bitumen can be evaluated on a more quantitative basis.

ACKNOWLEDGEMENTS

We thank the National Research Council of Canada for financial support. We are also indebted to Dr. E.M. Lown for carefully reading the manuscript.

REFERENCES

1. E.J. Gallegos, *Anal. Chem.*, **43**, 1151 (1971).
2. P.C. Wszolek, E. Gelpi, and A.L. Burlingame, *Advances in Org. Geochemistry*, 1971, p. 229, Pergamon Press, Oxford (1972).
3. W.K. Seifert, E.J. Gallegos, and R.M. Teeter, *Angew. Chem., Int. Ed.*, **10**, 747 (1971).
4. D.W. Westlake, A. Jobson, R. Phillippe, and F.D. Cook, *Can. J. Microbiol.*, **20**, 915 (1974).
5. M.L. Selucky, Y. Chu, T. Ruo, and O.P. Strausz, *Fuel*, in print.
6. M.T.J. Murphy in "Organic Geochemistry", G. Eglinton and M.T.J. Murphy, Eds., Springer-Verlag, New York, N.Y., 1969, p. 74-85.
7. A. Ensminger, Ph.D. Thesis: Triterpenoides du schiste de Messel, L. Pasteur University, Strassbourg, 1974.
8. B. Balogh, D.M. Wilson, P. Christiansen, and A.L. Burlingame, *Nature*, **242**, 603 (1973).
9. P. Engelhardt, paper presented at 11th International Symposium on Advances in Chromatography, Houston, Texas, November, 1976.

APPENDIX

Experimental Details for Figures 1-17:

- Figure 1 - Gas chromatographic analysis of saturate fractions eluted from alumina. Left: 6' x 1/8" column, 3% Poly S-179 phase on Chromosorb W-AW (100/120 mesh); T_1 220, T_2 320°C; t_1 5 min., rate 4°/min., F 32, Attn. 256x. Right: 6' x 1/8" column, 3% Dexsil 300 phase on Chromosorb W-AW (100/120 mesh); T_1 220, T_2 360°C; t_1 1 min., rate 6°/min., F 21 ml/min., Attn. 256x. STD: mixture of n-alkanes C_{11} , C_{13} , C_{15} , C_{19} , C_{20} , C_{22} , C_{24} , C_{28} , C_{32} and C_{36} in n-hexane.
- Figure 2 - HPLC test for the presence of monoaromatics in the saturate fractions eluted from alumina. Column: 70 cm x 1/4" alumina Woelm + 3% H₂O, particle size 18-32 μ m. Eluent: n-heptane, RI detector, P 220 psi, F = 6 ml/min.
- Figure 3 - Mass chromatograms (m/e 191, m/e 217) of the thiourea non-adduct of the saturate fraction #7 from alumina. Column: 6' x 1/8", 3% Dexsil 300 on Chromosorb W-AW (100/120). Conditions same as in Figure 1. GC/MS system: AEI MS-12 interfaced to an HP-5830A gas chromatograph via a heated transfer line and a Watson-Biemann type separator. Data system Nova DS-50.
- Figure 4 - a) GPC separation of fraction #5 from alumina.

b) GPC separation of fraction #6 from alumina.
c) GPC separation of fraction #7 from alumina.
Columns: 60 Å, 100 Å, 200 Å and 500 Å Styragel (Waters Assoc.) particle size 37-75 μm, 4' x 3/8" each, eluent methylene chloride, RI and UV (254 nm) detectors, F = 3 ml/min.

Figure 5 - Gas chromatograms of selected GPC fractions derived from fraction #5 from alumina.

Figure 6 - Gas chromatograms of selected GPC fractions derived from fraction #6 from alumina.

Figure 7 - Gas chromatograms of selected fractions derived from fraction #7 from alumina. All chromatograms in Figures 5-7 were run on a 3% Dexsil 300 column, conditions as in Figure 1.

Figure 8 - Preparative gas chromatogram of the hopane concentrate fraction. Column: 8' x 1/4", 3% Dexsil 300, TCD, isothermal 300°C, F = 60 ml/min. Cutting points are marked in the chromatogram.

Figure 9 - GC test of the purity of the isolated hopanes. Column same as in Figure 1, isothermal 300°C, F = 20 ml/min.
2 = C₂₇ hopane, 4 = C₂₉ hopane, 5 = C₃₀ hopane.

Figure 10- a) High-resolution mass spectrum of C₂₉ hopane.
b) High-resolution mass spectrum of C₃₀ hopane.
Instrument: AEI MS-50, 70 eV, direct probe.

Figure 11- ¹³C n.m.r. chemical shift line diagrams of C₃₀ hopane from Athabasca bitumen and published⁸ spectra.

Figure 12- Mass chromatograms of GPC fraction #9 from alumina fraction #7.

Figure 13- Gas chromatogram of the hopane series on a SCOT column (N ~ 28,000) coated with OV-101 on Silanox-101, 0.2 μl sample, Attn. 128x; T₁ 200°C, T₂ 280°C; t₁ 10 min., rate 2°/min.

Figure 14- High-resolution GC of the hopane series on a WCOT column (N ~ 80,000), 0.2 μl sample, split ratio approx. 1:10, Attn. 16x, isothermal 280°C.

Figure 15- Mass chromatograms of GPC fraction #13 from alumina fraction #6. Column type and conditions as in Figure 1.

Figure 16- Preparative HPLC of the total saturate fraction from the Athabasca bitumen. Column: 4' x 2 cm i.d., alumina 37-63 μm (fully activated), eluent n-pentane; F = 18 ml/min., RI detector.

Figure 17- Gas chromatograms of fractions I and II from Figure 16 on a SCOT column (OV-101, Silanox-101); sample 0.2 μl.

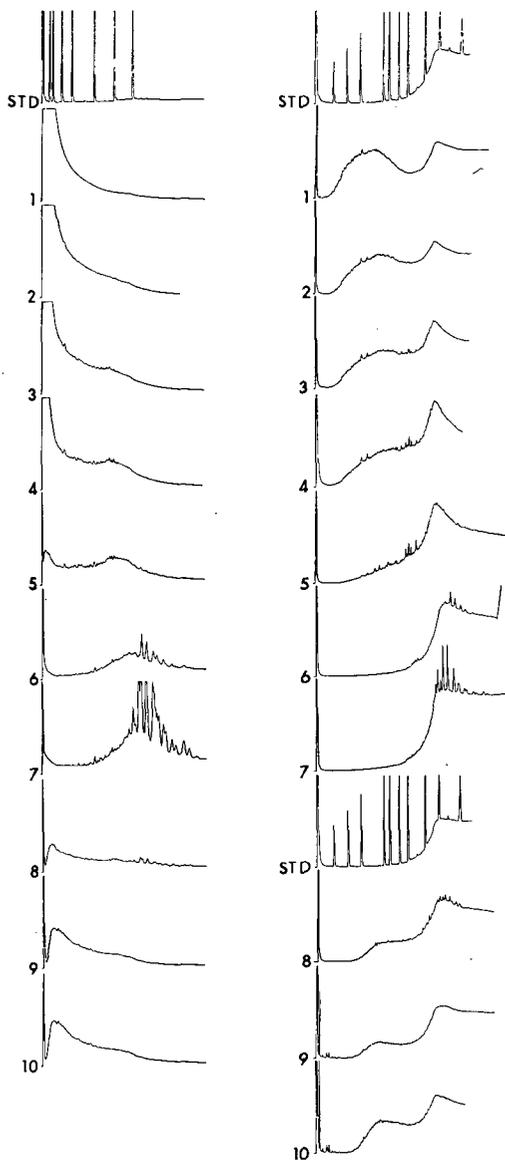


Fig. 1 - Gas chromatographic Analysis of saturate fractions eluted from alumina

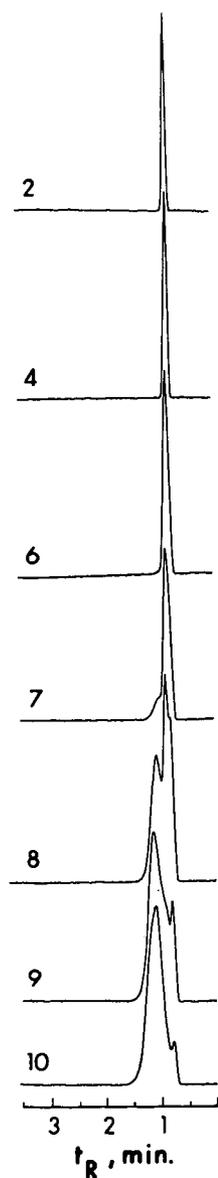


Fig. 2 - HPLC test for the presence of monoaromatics in the saturate fractions from alumina

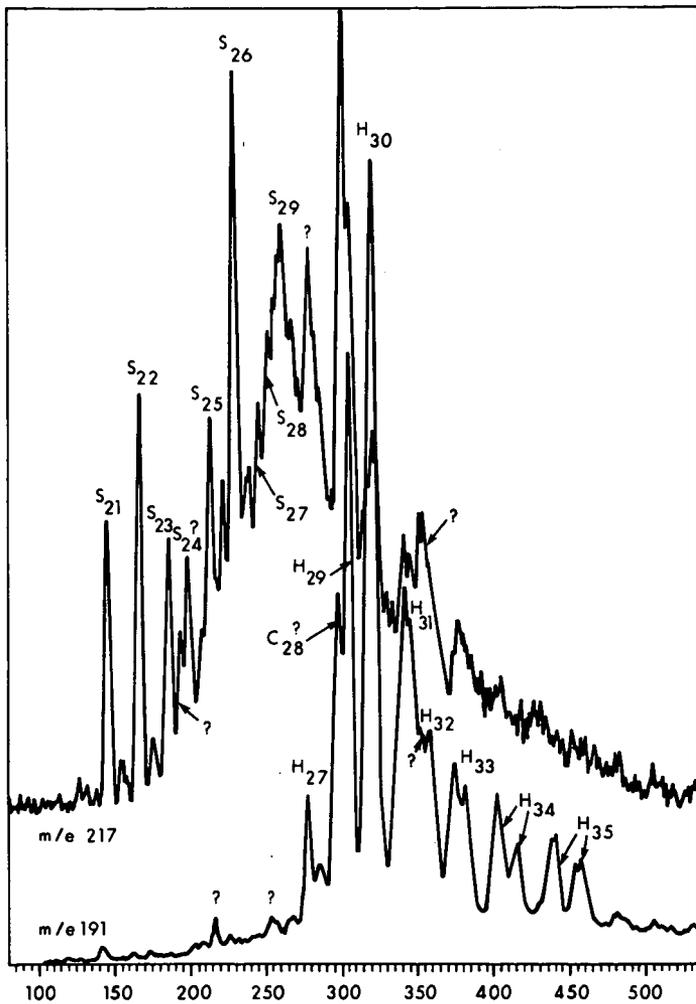


Fig. 3 - Mass chromatograms of the thiourea non-adduct of the saturate fraction #7 from alumina

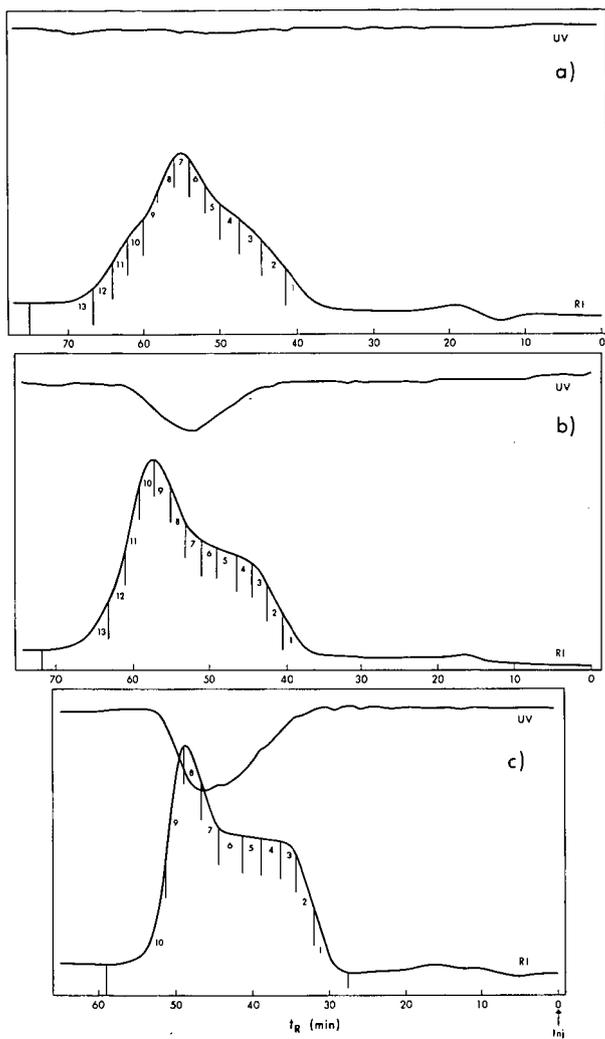


Fig. 4 - GPC separations of fractions #5 (a), #6 (b) and #7 (c) from alumina

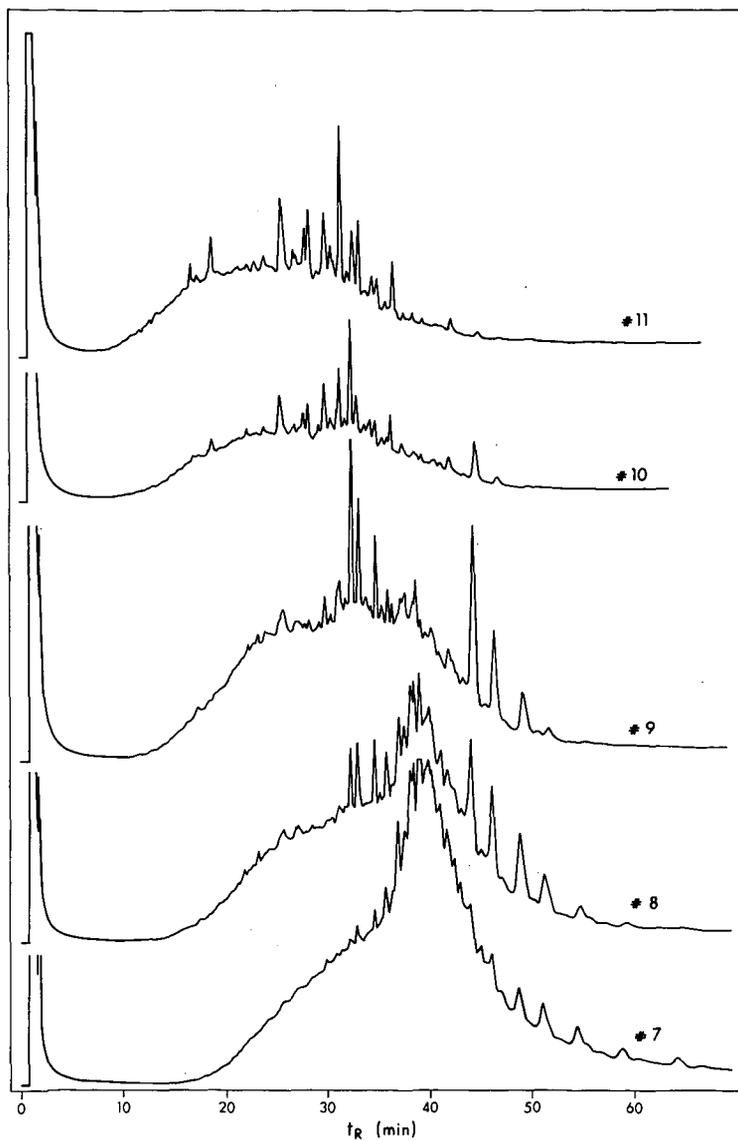


Fig. 5 - Gas chromatograms of selected GPC fractions derived from fraction #5 from alumina

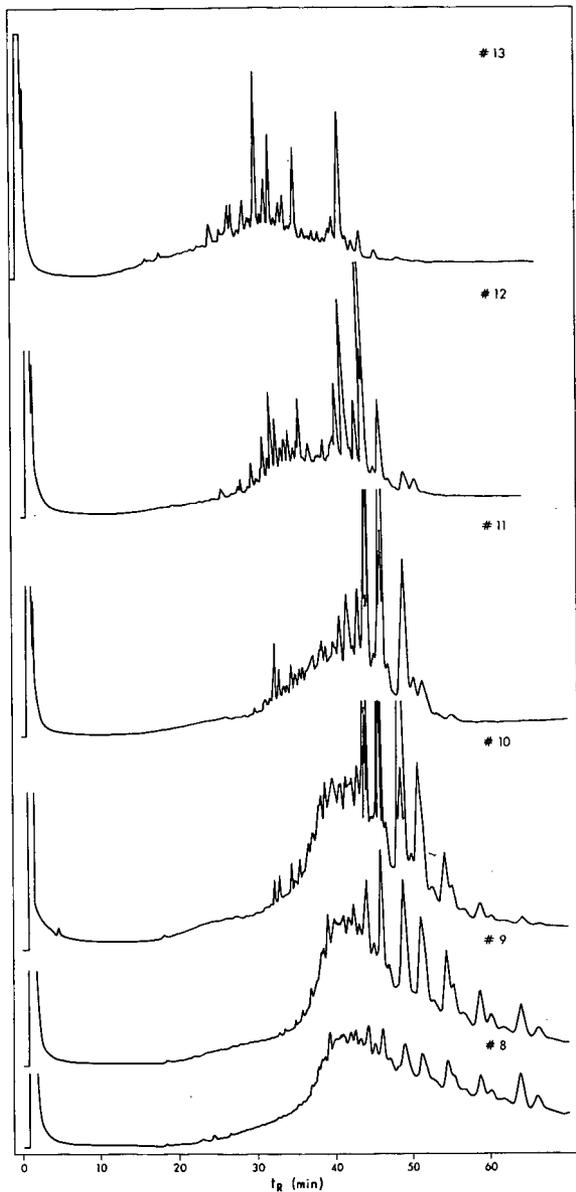


Fig. 6 - Gas chromatograms of selected GPC fractions derived from fraction #6 from alumina

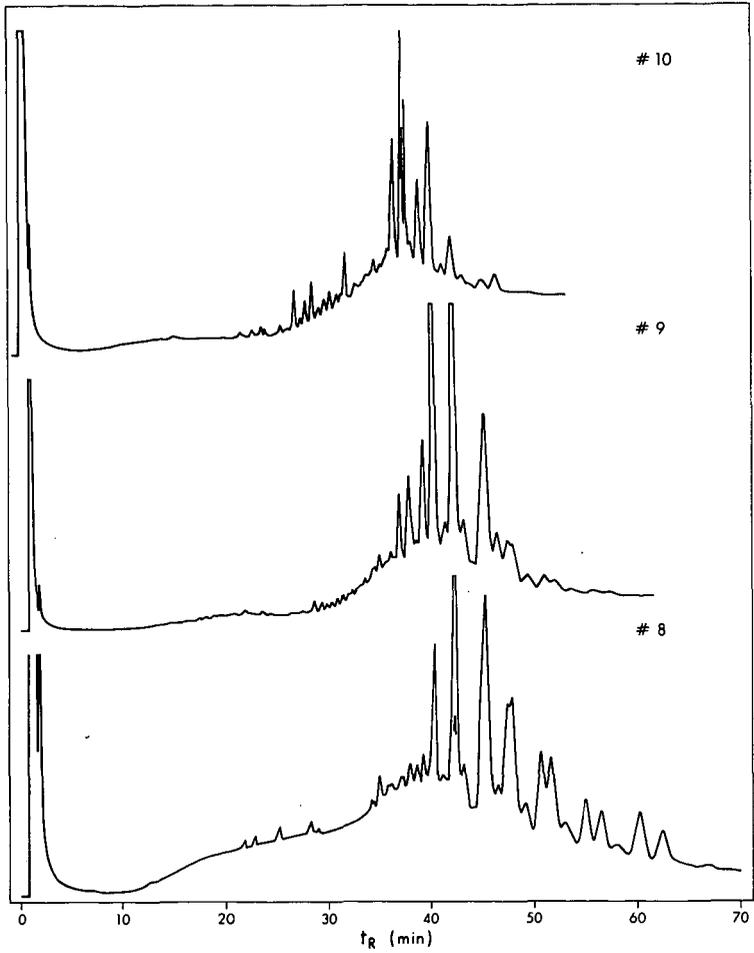


Fig. 7 - Gas chromatograms of selected GPC fractions derived from fraction #7 from alumina

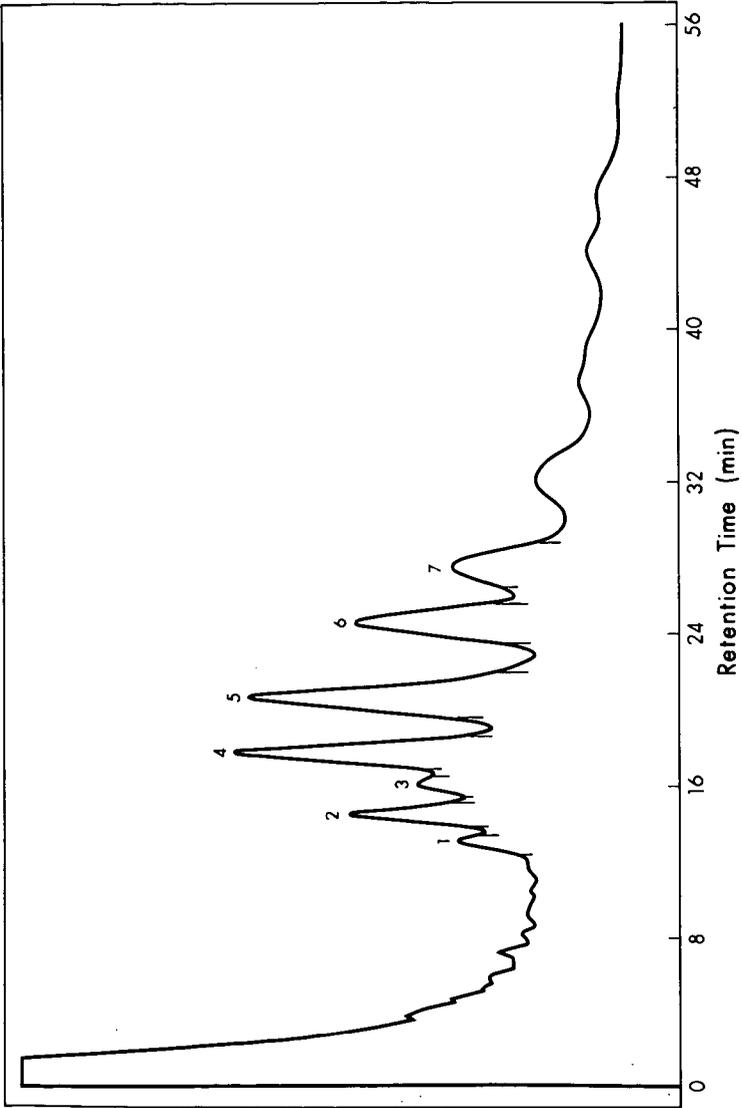


Fig. 8 - Preparative gas chromatogram of the hopane concentrate fraction

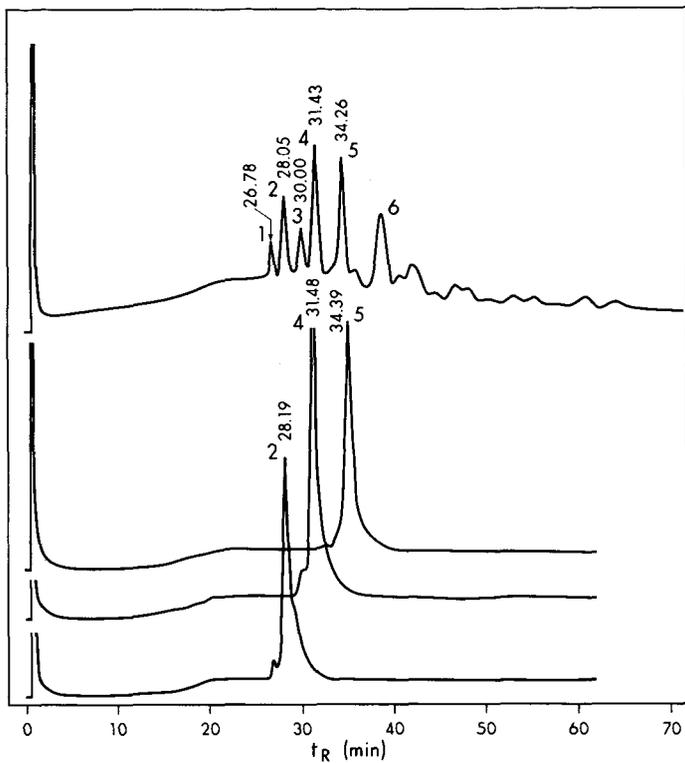


Fig. 9 - GC test of the purity of the isolated hopanes
 2 = C₂₇ , 4 = C₂₉ and 5 = C₃₀ hopane

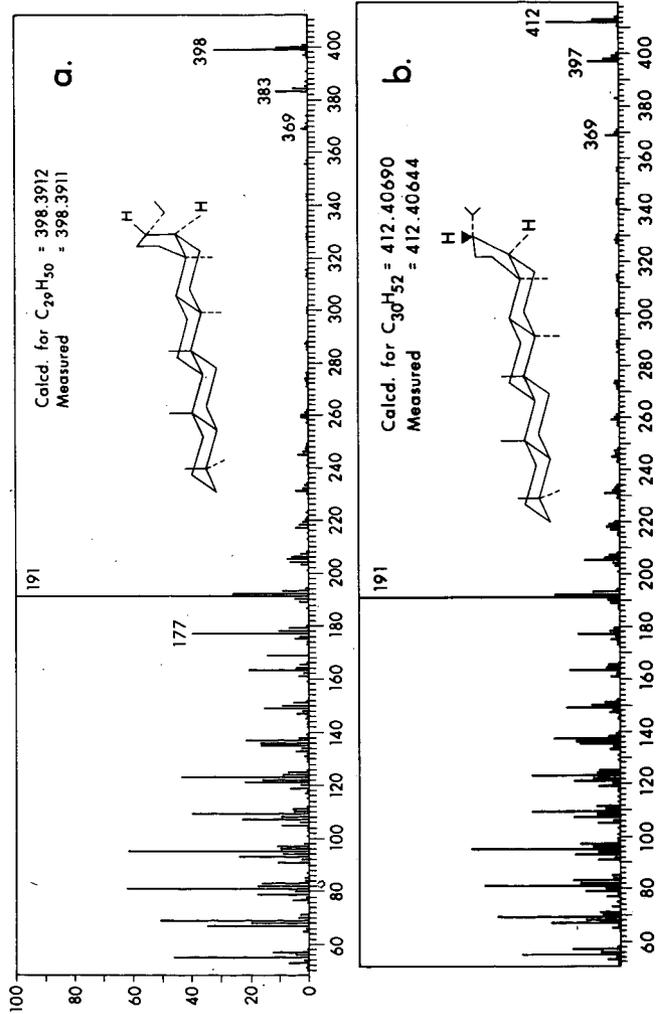


Fig. 10 - High-resolution mass spectra of C_{29} (a) and C_{30} (b) hopanes

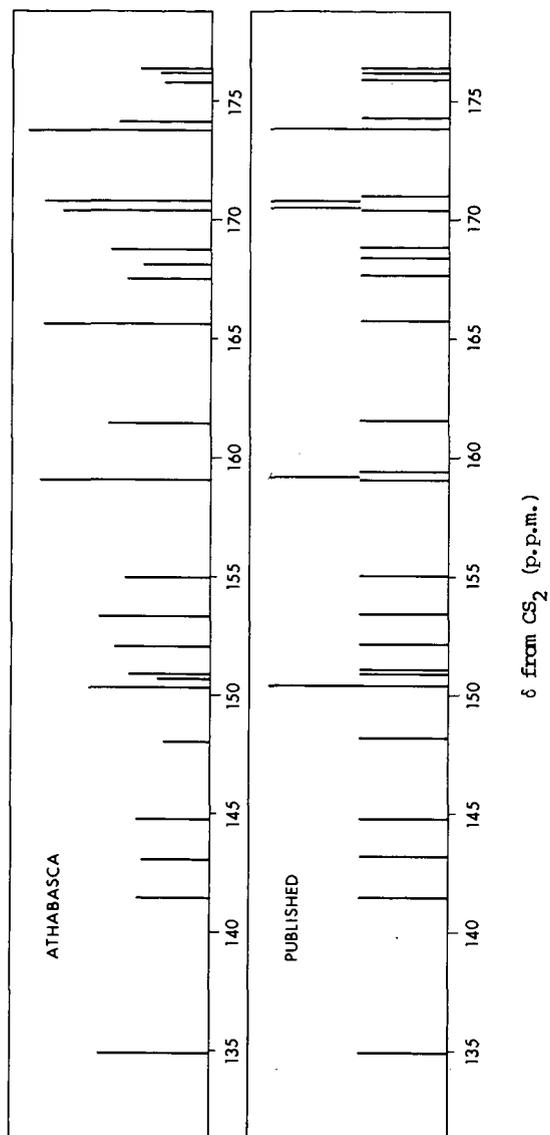


Fig. 11 - ¹³C n.m.r. chemical shift line diagrams of C₃₀ hopane from Athabasca bitumen and published⁸ spectra

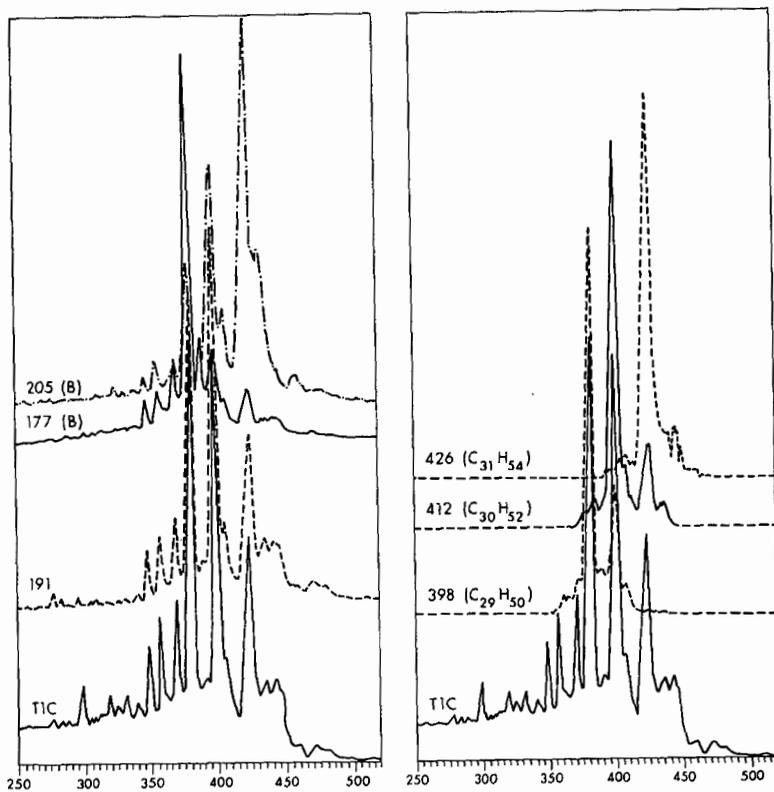


Fig. 12 - Mass chromatograms of GPC fraction #9 from alumina fraction #7

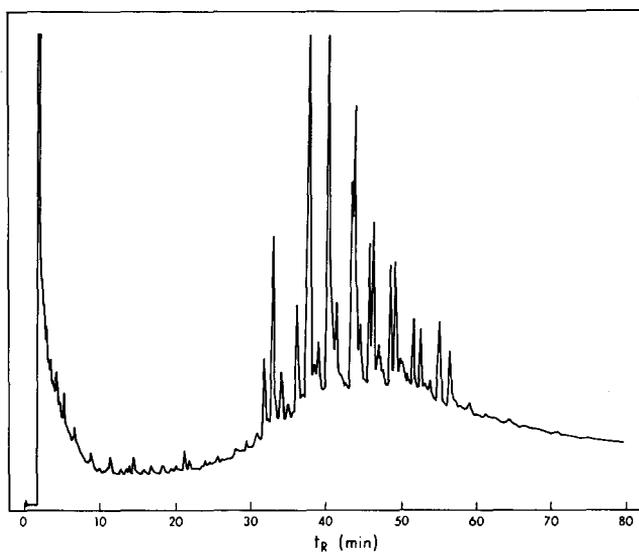


Fig. 13 - GC of the hopane series on a SCOT column

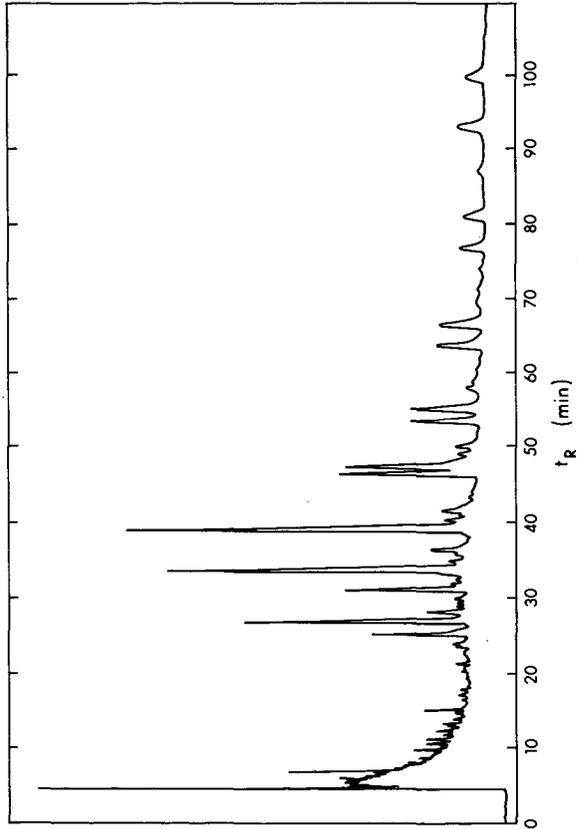


Fig. 14 - High-resolution GC of the hopane series on a WCOT column

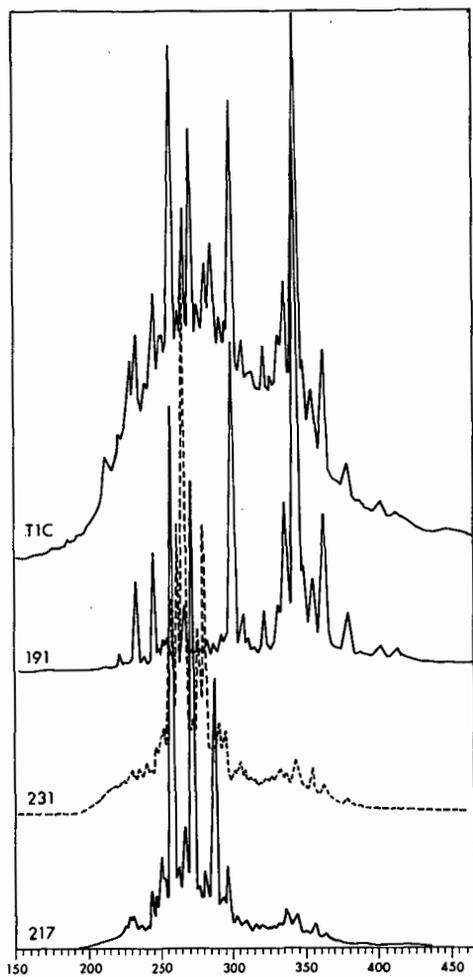


Fig. 15 - Mass chromatograms of GPC fraction #13 from alumina fraction # 6

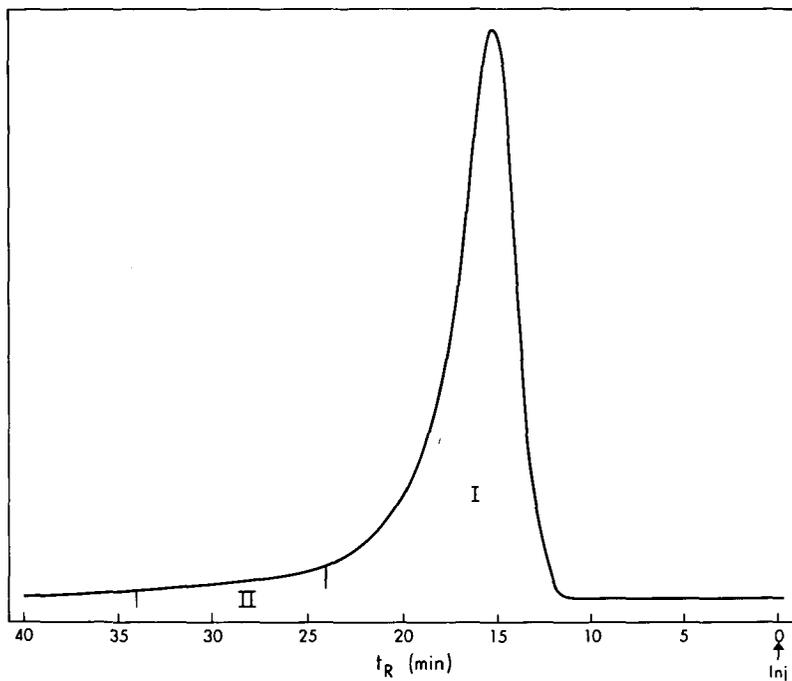


Fig. 16 - Preparative HPLC of the total saturate fraction from the Athabasca bitumen

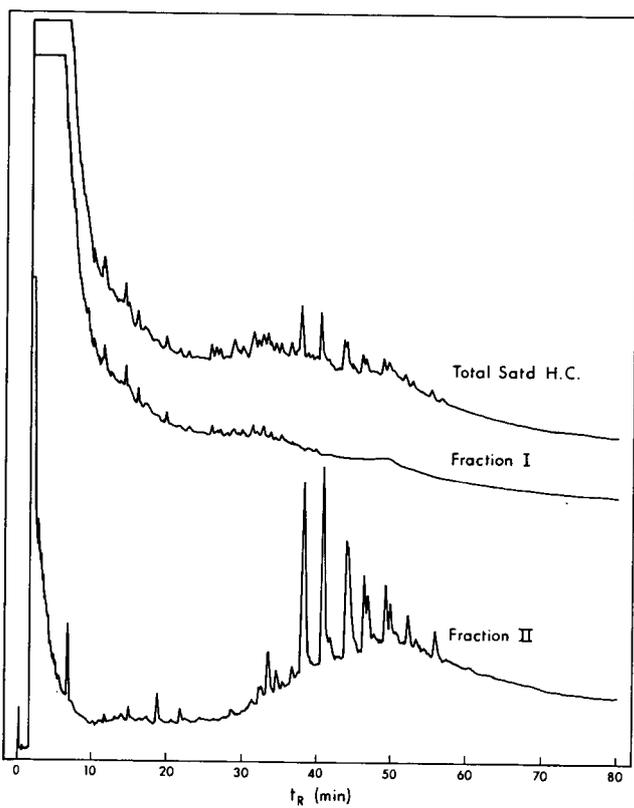


Fig. 17 - GC of fractions I and II from Fig.16 on a SCOT column