

Resolving the 'Hump'

Paul A. Sutton, Steven J. Rowland* and C. Anthony Lewis

<http://www.pegg.org.uk>

Petroleum and Environmental Geochemistry Group, Department of Environmental Sciences, University of Plymouth, Drake Circus, Plymouth, Devon, PL4 8AA, United Kingdom



Summary

Some individual hydrocarbons have been resolved from the unresolved complex mixture (UCM) of hydrocarbons of a biodegraded crude oil following preparative open column chromatography, preparative HPLC and preparative GC on two stationary phases. One of these compounds from the UCM was identified by electron impact ionisation GC-MS as a novel C₂₆ 17-desmethyl triaromatic steroid (ca 4 μg g⁻¹ whole oil). If this concentration is typical of the proportion of UCM represented by such compounds a very approximate estimate suggests that the whole oil studied contains maybe 250,000 compounds, indicating that oil UCMs are amongst the most complex mixtures of organic compounds on Earth. The above approach can lead to the identification of novel compounds which may have considerable geochemical, petrochemical and environmental significance, and will be applied to further UCM hydrocarbon fractions.

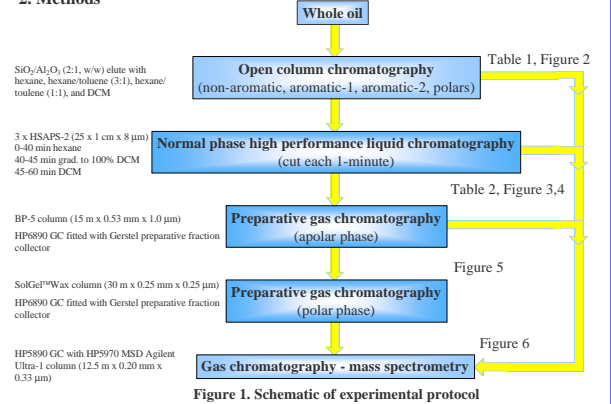
1. Introduction

- A large proportion of liquid petroleum remains a mystery [1]
- Relatively few oil hydrocarbons are separable by gas chromatography (GC) into individual, identifiable compounds
- Many appear as unresolved complex mixtures (UCMs) or GC 'humps' [e.g. 2]
- UCMs particularly obvious when biodegraded or chemically treated (e.g. by clathration) oils are examined by GC
- A lack of knowledge of detailed UCM composition;
 - hinders development of theories of the origin of petroleum
 - restricts prediction of the effects of refinery processing of crude oils
 - may limit the assessment of the environmental effects of oil residues in the environment [e.g. 3].
- Variety of techniques used to try to identify some of the components within oil UCMs;
 - studies of oxidised UCMs [1, 3-9]

- multidimensional GC [10, 11]
 - statistical deconvolution methods [12 - 14]
 - mass fragmentography and selected ion monitoring in GC-MS [e.g. 5 - 8]
- However, these techniques usually leave most of the UCM hydrocarbons unidentified.
- proposed structures of UCM compounds
 - non-aromatic (e.g. 'T'-branched alkanes [2,14])
 - aromatic hydrocarbons (e.g. substituted tetralins [2,15,16]).

The present study used open column chromatography, normal phase high performance liquid chromatography (HPLC) and preparative capillary GC on two stationary phases, to attempt to separate some individual aromatic hydrocarbons from the UCM of a biodegraded crude oil.

2. Methods



- UCM from Aromatic-1 fraction of TJP using normal phase HPLC (254 nm)
- 1-minute cuts from HPLC analysed by GC-MS
- Absorbance detection (254 nm) not quantitative
- Re-run of 1-minute cuts on HPLC - Gaussian peaks
- Double bond equivalents (DBE) [17] estimated from GC-MS mass spectra and confirmed using standards
- > alkyl substitution - shorter RT [18]

Table 2. HPLC retention times of standard compounds

Compound	DBE	RT
1-phenyldecane	4	26.05
1-(3'-methylbutyl)- 7-CHT	6	27.07
benzene	4	27.96
1-n-nonyl-7-CHT	6	28.23
1-n-propyl-7-CHT	6	29.07
1-methyl-7-CHT	6	29.51
tetralin	5	29.86
6-CHT	6	30.02
indene	6	36.73
1-n-nonyl-cyclohexyl-naphthalene	8	37.26
4-pentylbiphenyl	8	39.39
biphenyl	8	41.72
naphthalene	7	42.50
phenanthrene	10	64.15
anthracene	10	67.33

NB. CHT = cyclohexyltetralin

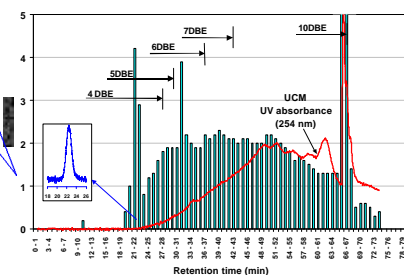
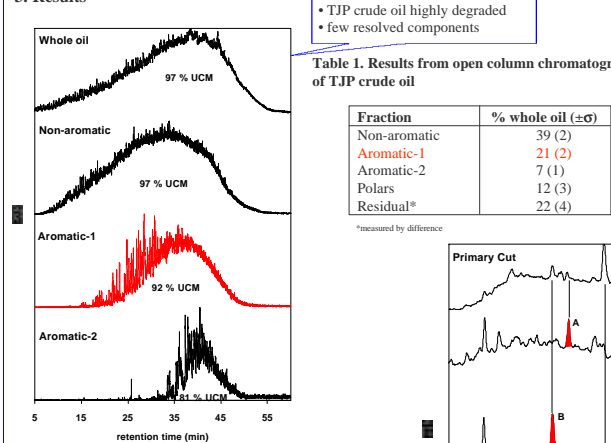


Figure 3. Normal phase HPLC UV chromatogram (254 nm, red line) of TJP aromatic-1 fraction and gravimetry of collected fractions. Re-run of 22 - 23 minute fraction shown inset. Latest retention times of each DBE integer indicated by arrows.

3. Results



- TJP crude oil highly degraded
- few resolved components

Table 1. Results from open column chromatography of TJP crude oil

Fraction	% whole oil (±σ)
Non-aromatic	39 (2)
Aromatic-1	21 (2)
Aromatic-2	7 (1)
Polars	12 (3)
Residual*	22 (4)

*measured by difference

- Increasing GC resolution with HPLC retention time
- Ion chromatograms (*m/z* 217, 231, 245) revealed presence of triaromatic steroids (TAS) [19]
- Use of *m/z* 217 for TAS acceptable because steranes in non-aromatic fraction (Figure 2) [20]
- 54 - 55 and 55 - 56 minute fraction subjected to preparative GC (apolar and polar phases)

- Primary (apolar) cut 8% resolved
- Secondary (polar) cuts (fractions F1 - F4) 28 - 50% resolved
- Collected fractions analysed by GC-MS
- Estimated peak A comprises 4 μg g⁻¹ whole oil, peaks B-D 5 - 7 μg g⁻¹ whole oil
- If these concentrations represent 'average' then estimated that this oil contains some 250,000 compounds
- EI mass spectra of peaks B, C and D consistent with those of well-known C₂₆ - C₂₈ 17-methyl TAS [19,21]

- Peak A base ion consistent with Diels' hydrocarbon [22]
- Dominant molecular ion *m/z* 344 in EI mass spectrum of peak A (Figure 6) consistent with those of well-known C₂₆ 17-desmethyl TAS

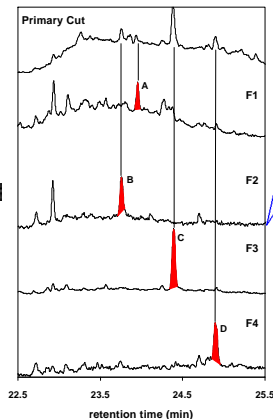


Figure 5. Partial reconstructed ion chromatograms from the GC-MS analysis of primary (apolar) and secondary (polar) preparative GC isolation of triaromatic steroids from the 54 - 56 minute HPLC cuts

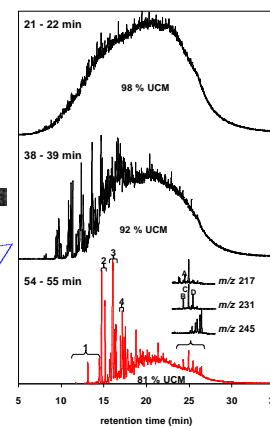


Figure 4. Typical reconstructed ion chromatograms of 1-minute HPLC cuts (1 = C₀ - C₂ fluorenes [9 DBE]; 2 - 4 = C₁, C₂, C₃ dibenzothiophenes, respectively [9 DBE]; *m/z* 217 = desmethyl TAS; *m/z* 231 = methyl TAS; *m/z* 245 = dimethyl TAS [11 DBE])

4. Conclusions

- This chromatographic approach can be used to resolve components from an UCM
- The EI mass spectrum of a novel C₂₆ 17-desmethyl triaromatic steroid has been obtained for the first time
- Whole oil UCM estimated to contain 250,000 compounds
- Oil UCMs amongst most complex mixtures of organic compounds on Earth

5. Further Work

This approach will be applied to further HPLC fractions including the toxicologically significant ones [23]

5. Acknowledgements

We wish to thank the Natural Environmental Research Council for financial assistance (Grant No. GR3/13184)

6. References

[1] K.H. Ahlgren, M.M. Boudarynski, Composition and Analysis of Heavy Petroleum Fractions, Marcel Dekker, New York, 1993. [2] M.A. Gough, S.J. Rowland, Nature, 344 (1990) 648. [3] S. Rowland, P. Donkin, E. Smith, E. Waage, Environ. Sci. Technol., 35 (2000) 2640. [4] B. Wartos, Ph.D. Thesis, Curtin University of Technology, Perth, Australia, 1999. [5] B. Wartos, R. Alexander, R.I. Kagi, Org. Geochem. 30 (2000) 1255. [6] S. Killips, M.A.H.A. Al-Jubouri, Org. Geochem. 15 (1990) 147. [7] A.T. Revell, Ph.D. Thesis, University of Plymouth, 1992. [8] J.B. Phillips, J. Beens, J. Chromatogr. A 856 (1999) 331. [9] J. Blomberg, P.J. Schoenmakers, J. Beens, R. Tijssen, J. High Resolut. Chromatogr. 20 (1997) 539. [10] M. van Duuren, J. Beens, J. Reijnen, P. Lipman, C. Cunnery, J. Blomberg, J. High Resolut. Chromatogr. 23 (2000) 507. [11] S. Dagun, J. Chromatogr. A 869 (2000) 229. [12] C. Denis, P. Houdouma, R.G. Breerton, Analyst, 125 (2000) 267. [13] W.G. Peol, J.W. de Leeuw, B. van de Graaf, J. Mass Spectrom. 32 (1997) 438. [14] B. Wartos, R. Alexander, R.I. Kagi, Org. Geochem. 27 (1997) 465. [15] B. Wartos, R. Alexander, R.I. Kagi, Org. Geochem. 30 (1999) 1255. [16] K.V. Thomas, Ph.D. Thesis, University of Plymouth, 1995. [17] S.D. Killips, J.W. Readman, Org. Geochem. 8 (1985) 247. [18] M.S. Ahlberg, J. Chromatogr. 644 (1993) 253. [19] K.E. Peters, J.M. Moldovan, The Biomarker Guide, Prentice-Hall Inc., N.J., 1993, p. 245. [20] A.S. Mackenzie, C.F. Hoffman, J.R. Maxwell, Geochim. Cosmochim. Acta 45 (1981) 1345. [21] L.L. Tikles, C. Djerosi, J. Am. Chem. Soc. 91 (1969) 5017. [22] S.J. Rowland, K. Aureskjold, G. Xie, Xie, A.G. Douglas, Org. Geochem. 10 (1986) 1033. [23] E.L. Smith, P. Donkin, S.J. Rowland, Environ. Toxicol. Chem. 20 (2001) 2428.

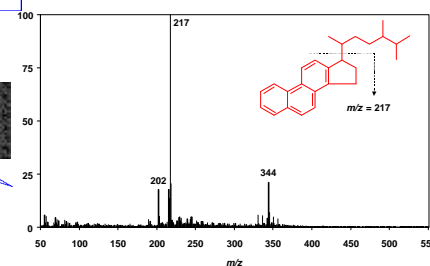


Figure 6. EI mass spectrum of Peak A a novel C₂₆ 17-desmethyl triaromatic steroid