

HYDROPYROLYSIS AS A PREPARATIVE METHOD FOR THE COMPOUND SPECIFIC CARBON ISOTOPE ANALYSIS OF STEROIDS

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The ability to accurately determine the carbon isotopic composition of steroids by standard gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) techniques would be of great benefit for a variety of environmental and biological science applications. However, steroids in their natural form exhibit poor chromatographic resolution, while derivatisation adds extra carbon atoms thereby corrupting the starting stable isotopic composition of the target molecules (Wolthers and Kraan, 1999).

This study describes the application of hydroxylation to the defunctionalisation of individual steroids to yield their corresponding hydrocarbons, thus retaining the carbon skeleton intact while improving chromatographic resolution, allowing for the faithful measurement of carbon isotope ratios. Hydroxylation, which involves the catalytic addition of hydrogen to the carbon skeleton under a high hydrogen gas pressure (15 MPa), was originally developed as a method for the analysis of covalently bound biomarkers in crude oils and source rocks (Love et al., 1995), and has been successfully used to defunctionalise fatty acids prior to GC-C-IRMS analysis (Sephton et al., 2005a).

The product recovered after the hydroxylation of 5 α -cholestanol (Fig. 1a) is composed largely (>95%) of 5 α -cholestane. Very small amounts of 5 β -cholestane and two isomers of unsaturated cholestene were also detected. The results illustrate that, in addition to defunctionalising aliphatic chains as demonstrated for carboxylic acids (Sephton et al., 2005a), hydroxylation also efficiently eliminates exocyclic oxygen-containing functional groups. For cholesterol, effective hydrogenation would be expected to produce two cholestane isomers (5 α and 5 β) owing to the non-selective nature of the hydrogenation reaction for alkene moieties adjacent to positions where rings join. However, GC/MS analysis of the products (Fig. 1b) shows that extensive rearrangement occurs with the hydroxylation procedure giving, in addition to the two expected cholestane isomers, four diasteranes and an unresolved complex mixture, comprising other diasteranes, cholestenes and diasterenes.

Comparison of the carbon isotopic determinations for untreated 5 α -cholestanol and cholesterol (as determined by combustion-IRMS), and the products of hydroxylation by GC-

C-IRMS (Fig. 1), indicate that the isotopic composition of the processed sample is consistent with that of the starting material. The difference between the two measurements is within the error of the instruments and it appears that no isotopic effects are associated with conversion from the functional steroid to their hydrocarbon counterpart.

Poor selectivity was observed for other multifunctional or unsaturated steroids such as testosterone and androstanediol. Therefore, developments to the catalyst system and temperature regime are ongoing in order to promote low temperature hydrogenation, and so eliminate rearrangements caused by the migration of the carbon double bonds, thereby achieving the highly selective defunctionalisation and accurate isotopic characterisation of these analytically challenging molecules.

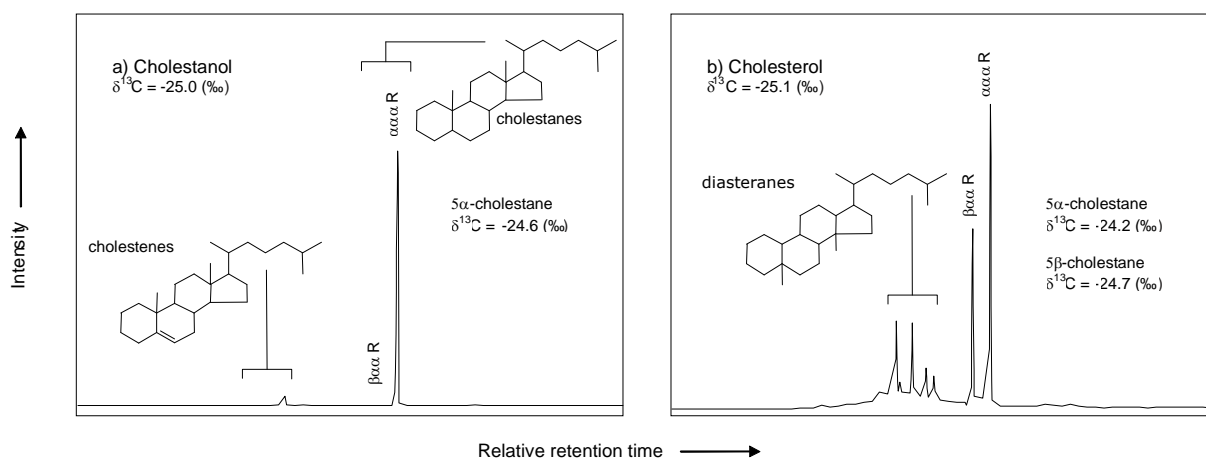


Figure 1. GC-MS traces of the hydroxyprolysis products of (a) cholestanol and (b) cholesterol (modified from Sephton et al., 2005b).

REFERENCES

- Wolthers, B.G. and Kraan, G.P.B. (1999) Clinical applications of gas chromatography and gas chromatography–mass spectrometry of steroids. *Journal of Chromatography A* **843**, 247-274.
- Love, G.D., Snape, C.E., Carr, A.D. and Houghton, R.C. (1995) Release of covalently-bound alkane biomarkers in high yields from kerogen via catalytic hydroxyprolysis. *Organic Geochemistry* **23**, 981-986.
- Sephton, M.A., Meredith, W., Sun, C. and Snape, C.E. (2005a) Hydroxyprolysis as a preparative method for the compound-specific carbon isotope analysis of fatty acids. *Rapid Communication in Mass Spectrometry* **19**, 323-325.
- Sephton, M.A., Meredith, W., Sun, C. and Snape, C.E. (2005b) Hydroxyprolysis of steroids: a preparative step for compound-specific carbon isotope ratio analysis. *Rapid Communication in Mass Spectrometry* **19**, 3339-3342.