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Utility of 5 Å molecular sieves to measure carbon isotope ratios in lipid biomarkers

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Abstract

A procedure using 5 Å zeolite sorption to separate cyclic/branched organic compounds from the linear ones was developed and carbon isotopic fractionation effects were investigated in different families of compounds, e.g. within the hydrocarbon and alcohol compounds. The 5 Å sieve has a pore size such that only linear components can be incorporated into the pores whereas the cyclic/branched compounds are remaining free in the organic solution. The sorbed compounds were released from the molecular sieve with HF and solvent extracted with hexane. The method enables the isolation of linear saturated classes, such as *n*-alkanes and *n*-fatty alcohols from branched/cyclic compounds without isotopic fractionation for compound-specific isotope analysis (CSIA) of $\delta^{13}C$. However, alkene hydrocarbons, sterols and some aromatics were completely or partly degraded with the molecular sieve.

Keywords: Alkanes; Aliphatic hydrocarbons; Polycyclic aromatic hydrocarbons; Sterols; Alkanols; Alcohols; Carbon isotope ratios; Lipid biomarkers; Zeolite molecular sieve

1. Introduction

Zeolite molecular sieves are crystalline structures defined by large internal three-dimensional cavities where molecules can be sorbed. Generally, they contain silicon, aluminium and oxygen in their framework, and cations, water and/or other molecules within their pores. These cavities are interconnected by pore openings through which molecules can pass, and because of their crystalline nature, the pores and cavities are all precisely the same size. Depending on the size of the openings, they can incorporate molecules readily, slowly, or not at all, thus functioning as molecular sieves – absorbing molecules of a certain size while rejecting larger ones. There are a number of different types commonly available [e.g. types 3 Å (3A), 4 Å (4A), 5 Å (5A), 10X, ZSM-5, mordenite, Ultrastable-Y, NaX), each with its own inherent pore size.

For many decades, organic geochemists have used synthetic zeolite molecular sieves of 5 Å to separate straight chain from other saturated hydrocarbon components in saturated fractions from sedimentary organic matter and oils [1–5]. The main purpose was to separate a complex mixture into subfractions of well resolved compounds to provide a better fingerprinting of the oil. Nowadays, the compound-specific isotope analysis (CSIA) is a powerful tool to characterize the origin of the hydrocarbons [3,6–13], but it requires a high chromatographic resolution of the individual compounds [14–18]. Molecular sieves might be used to reduce the complexity of such mixtures and enable more reliable analysis for CSIA. Nevertheless, any carbon isotopic fractionation effects occurring during the sample procedure should be first assessed. Sample preparation techniques involving chromatographic techniques, such as HPLC, have shown to induce important isotope fractionation, requiring quantitave collection for accurate isotope ratio determination [19,20]. Size-exclusion techniques including urea adduction and molecular sieves showed, however, no measurable isotope fractionation effect and appeared to be an inexpensive chromatographic technique to isolate the resolved aliphatic hydrocarbons [1,21,22]. Hence, the improved separation properties of the molecular sieves merit further study and we have also explored the use of zeolite molecular sieves to separate other compounds than

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aliphatic hydrocarbons, such as polycyclic aromatic hydrocarbons and alcohols.

The goal of this study was to determine if the 5 Å molecular sieve procedure was able to separate the linear from the branched/cyclic compounds within different families of compounds and determine if there is any isotopic fractionation effect for CSIA of $\delta^{13}C$. Aliphatic standards containing cyclic, branched, linear and alkene compounds; polycyclic aromatic hydrocarbons (PAHs) and alcohols containing linear and steroid compounds were used to investigate the utility of the procedure.

2. Experimental

2.1. Reagents and materials

Solvents were of high purity pesticide quality (Burdick and Jackson Labs., Muskegon, MI, USA). Sodium sulfate anhydrous, sodium chloride, bis(trimethylsilyl)trifluoroacetamide (BSTFA), and 48% HF were supplied by Merck (France). Sodium sulphate used for drying organic extracts was baked at 450 °C overnight before use in order to remove any traces of organic contaminants. Washed molecular sieves with a diameter pore size of 5 Å and a standard bead size of 60–80 mesh was obtained from Alltech Europe (Eke, Belgium) and activated overnight at 450 °C. Molecular sieves possess a macropore structure of high capacity with an active internal surface of the order of 500–900 $m^2\,g^{-1}$. Analyte/zeolite ratios of the order of 1/20 were shown to be sufficient to incorporate compounds in the zeolite without any saturation of their cavities [2].

All standards were of the highest purity commercially available: *n*-alkanes, pristane, phytane and PAHs were obtained from J.T. Baker (Noisy le Sec, France), perdeuterated alkanes and perdeuterated PAHs from Cambridge Isotope Labs. (USA), Friedelin Tech from Aldrick (Saint Quentin Fallavier, France), squalane, cholest-5-en-3β-ol (cholesterol) and 24-ethylcholest-5-en-3β-ol (sitosterol) were purchased from Sigma (Saint Quentin Fallavier, France), 5β-cholestan-3β-ol (coprostanol) from Research Plus (Bayonne, NJ, USA), 5α-androstan-3β-ol, 24α-methylcholest-5-en-3β-ol (campesterol) and 5α-cholestan-3β-ol (cholestanol) from Steraloids (New Port, RI, USA), lycopane, 5β(H) cholane, 17β(H), 21β(H) hopane, hop-22(29)-ene (diploptene), β,β-carotane, from Chiron (Trondheim, Norway).

Stock individual standard solutions (0.8–1 $mg\,ml^{-1}$) were prepared by dissolving accurately weighed amounts of standards in isooctane and were stored at −4 °C. Working standard mixtures were obtained by further dilution of stock solutions with isooctane.

The saturated fraction of the IAEA-357 reference sediment and the alcohol fraction of the IAEA-408 reference sediment [23] were used as test samples for the present separation procedure. These fractions were obtained as described in the protocol of [24].

2.2. Isolation

One gram of activated molecular sieve was added to a 50 ml PTFE tube containing the organic extract (∼2 ml isooctane) and the mixture was left sealed overnight. A temperature of 100 °C was chosen based on the procedures of Ishiwatari et al. [3]. The isooctane solvent was recovered and the pellets were washed three times with 2 ml of hexane by ultrasonication and centrifugation at 4000 rpm for 5 min. The hexane washings were combined with the isooctane extract containing the excluded fraction with the cyclic/branched compounds.

After wash with *n*-hexane, the pellets containing the sorbed compounds were dissolved by slowly adding 2 × 3 ml HF solution and 5 ml saturated sodium chloride water. The released compounds were extracted with hexane (4 ml, 2 times and 3 ml, 1 time). The organic extract containing the included compounds was rinsed with 4 ml of saturated sodium chloride water and dried with activated sodium sulphate. The two organic fractions, the excluded and included fractions, were evaporated under a gentle nitrogen stream to 200 μl for GC analysis.

2.3. Derivatisation

Alcohols were treated with 200 μl BSTFA for 1 h at 70 °C to convert the hydroxyl group to their corresponding trimethylsilyl ethers. The derivatized extract was then evaporated to dryness under a nitrogen stream and redissolved in isooctane for injection on the gas chromatograph. No correction for the isotopic change introduced in the derivatisation of sterols and fatty alcohols was done because only relative variations between the processed and unprocessed standards were considered. Otherwise, the corrected value can be derived following the mass balance equation of Rieley [25].

2.4. GC and GC/MS analyses

Gas chromatography (GC) was performed with a Hewlett-Packard HP 5890 series II equipped with a flame ionization detection (FID) system, a split/splitless injector and an HP 7673 autoinjector. A 5% phenylmethylpolysiloxane fused silica capillary column was used, (DB5, 30 m × 0.25 mm I.D.; film thickness 0.25 μm; Agilent, Massy, France). Helium was the carrier gas (1.2 $ml\,min^{-1}$). The oven temperature was programmed from 60 °C (0.5 min hold) to 300 °C at 6 $°C\,min^{-1}$ and maintained at 300 °C for 30 min. The injector and detector temperatures were 270 and 320 °C, respectively. Confirmation of peak identity was obtained using a HP5890 series II GC system with mass spectrometric detection (GC/MS) (Hewlett-Packard 5889B MS "Engine") operated in the electron impact ionization mode and using a HP-5MS (cross-linked 5% phenyl-methylsilicone) column of 30 m × 0.25 mm I.D., 0.25 μm film thickness. The operating conditions were: mass range 50–550 u; electron energy 70 eV, transfer line temperature 280 °C; 0.9 $scan\,s^{-1}$. Recoveries of the different spiked standards were quantified relative to a friedelin GC internal standard.

2.4.1. Isotope-ratio-monitoring gas chromatography-mass spectrometry

Lipid biomarkers were analysed for their stable carbon isotope composition using an HP 5890 GC equipped with an HP