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Review

Recent advances in hopanoids analysis: Quantification protocols overview, main research targets and selected problems of complex data exploration

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ABSTRACT

Pentacyclic triterpenoids, particularly hopanoids, are organism-specific compounds and are generally considered as useful biomarkers that allow fingerprinting and classification of biological, environmental and geological samples. Simultaneous quantification of various hopanoids together with battery of related non-polar and low-molecular mass compounds may provide principal information for geochemical and environmental research focusing on both modern and ancient investigations. Target compounds can be derived from microbial biomass, water columns, sediments, coals, crude fossils or rocks. This create number of analytical problems due to different composition of the analytical matrix and interfering compounds and therefore, proper optimization of quantification protocols for such biomarkers is still the challenge. In this work we summarizing typical analytical protocols that were recently applied for quantification of hopanoids like compounds from different samples. Main steps including components of interest extraction, pre-purification, fractionation, derivatization and quantification involving gas (1D and 2D) as well as liquid separation techniques (liquid–liquid extraction, solid-phase extraction, planar and low resolution column chromatography, high-performance liquid chromatography) are described and discussed from practical point of view, mainly based on the experimental papers that were published within last two years, where significant increase in hopanoids research was noticed. The second aim of this review is to describe the latest research trends concerning determination of hopanoids and related low-molecular mass lipids analyzed in various samples including sediments, rocks, coals, crude oils and plant fossils as well as stromatolites and microbial biomass cultivated under different conditions. It has been found that majority of the most recent papers are based on uni- or bivariate approach for complex data analysis. Data interpretation involves number of physicochemical parameters and hopanoids quantities or given biomarkers mass ratios derived from high-throughput separation and detection systems, typically GC–MS and HPLC–MS. Based on quantitative data reported in recently published experimental works it has been demonstrated that multivariate data analysis using *e.g.* principal components computations may significantly extend our knowledge concerning proper biomarkers selection and samples classification by means of hopanoids and related non-polar compounds.

Contents

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1. Introduction

Hopanoids belongs to large family of triterpenoids containing five rings that form the chemical structure of hopane (Fig. 1) [1]. Pentacyclic triterpenoids are non-polar membrane components and were found in many microbial species. Particularly, they allow to adapt the bacteria organisms to extreme environmental conditions [2]. These steroids are considered to have the biochemical functions similar as cholesterol in eukaryotes [3], however, detailed role of hopanoids in bacterial physiology is still uncertain [4]. There are evidences that they may improve plasma membrane strength and rigidity, play critical roles in alteration of cell membrane permeability by reducing of proton and sodium leaks through lipid bilayers as well as allow microorganisms adaptation to extreme environmental conditions, including pH and temperature changes [5,6]. Similarly to different groups of lipids (*e.g.* fatty acids, ether lipids, carotenoids), hopanoids are considered as the diagnostic or fossil biomarkers and provide principal information for geochemical and environmental research focusing on both modern and ancient investigations [5]. Due to the fact that non-polar substances form the major fraction of marine and terrestrial sediments, it has been hypothesized that lipids, including hopanoids, are the most abundant organic compounds on the Earth [7]. Hopanoids are organism-specific compounds because they are predominantly present in aerobic bacteria, therefore, such low-molecular mass chemicals can be considered as practical indicators for aerobic microorganisms [8]. In spite of this observation, there are several contributions indicating that hopanoids can be also efficiently produced by selected anaerobic microorganisms [9–14]. Analysis of hopanoids derived biomarkers in water and sediments allows to understand the geological processes and organisms involved in methane cycling [15]. Hopanoids were identified as the biomarkers for extant or extinct life from the terrestrial fossil records and are considered as the probable life indicating organic molecules, particularly for a future robotic analytical exploration of Mars [16]. They can be used as the target biomarkers and fingerprints for characterization of the present ecosystems and their geological records.

Hopanoids are fairly non-homogeneous group of steroid-like compounds and their various structures have been observed and detected in modern as well as ancient marine/terrestrial sediments [5,17]. For example, the most commonly observed hopanoid, which is named diplopterol, is based on C_{30} pentacyclic skeleton (Fig. 2). Common derivatives containing C_{30} pentacyclic skeleton are 2-methyldiplopterol or neriifoliol (hopan-29-ol) as well as number of triterpenoids with a wide variety of structural modifications are based on fern-9(11)-ene (*e.g.* arundoin) or arbor-9(11)-ene (*e.g.* isoarborinol) [17]. C_{31}–C_{35} hopanes are named homo-, bishomo-, trishomo-, tetrakishomo-, and pentakishomohopanes, respectively. Number of structures are derived from C_{35} skeleton (bacteriohopanoids, homohopanoids), in which *n*-pentyl group is attached to the hopanoid carbon skeleton at the C_{30} position (Fig. 3) [5]. Hopanes are degradation products of bacteriohopanepolyols (BHPs) and it is widely accepted that most sedimentary hopanoids are degradation products of BHPs or are derived from the C_{30} hopanoids (diplopterol and diploptene) [7,18,19]. It has been found that detection of specific functional groups (including hydroxyl, amino and sugar groups) attached to *n*-pentyl group of bacteriohopanoids enables detailed diagnostic information, because different structures of hopanoids are present in diverse groups of aerobic microorganisms [20]. Tetrathymanol is precursor of gammacerane that is an example of pentacyclic triterpene (Fig. 4), which is constituent of sedimentary organic matter. Homohopanes play an important role in geochemical investigations and are often selected as the useful diagnostic biomarker indicators, particularly for proofing of the origin in oil spill analysis, oil waste analysis and analysis of airborne particulates [21]. Different reported hopanes structures include aromatic hopanoids (Fig. 5) or rearranged hopanes, in which the carbon skeleton is identical to the hopanes except that the methyl group is at position C-17, instead of position C-18 (Fig. 6) [22].

In this review, we predominantly focused on the basic analytical aspects associated with qualitative/quantitative protocols that are recently applied for hopanoids determination and fingerprinting from complex biological, environmental and geological

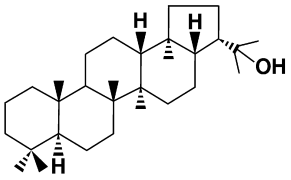

Fig. 2. Hopane derivative – diplopterol (hopan-22-ol).

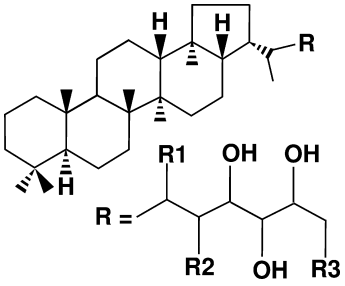

Fig. 3. Chemical structures of tetra- (R1,R2 = H), penta- (R1 = H, R2 = OH) and hexafunctionalised (R1,R2 = OH) bacteriohopanoids; R3 corresponds to range of functional groups including hydroxyl, amino and sugar groups (based on data provided by reference [5]).

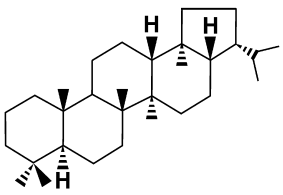

Fig. 1. Chemical structure of hopane.

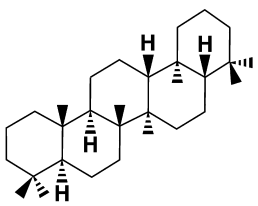

Fig. 4. Example of pentacyclic triterpene–gammacerane.