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Testing the preservation of biomarkers during experimental maturation of an immature kerogen

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Abstract: Lipid biomarkers have been extensively applied for tracing organisms and evolutionary processes through Earth's history. They have become especially important for the reconstruction of early life on Earth and, potentially, for the detection of life in the extraterrestrial realm. However, it is not always clear how exactly biomarkers reflect a paleoecosystem as their preservation may be influenced by increasing temperatures (T) and pressures (P) during burial. While a number of biomarker indices reflecting thermal maturity have been established, it is often less well constrained to which extent biomarker ratios used for paleoreconstruction are compromised by T and P processes. In this study we conducted hydrous pyrolysis of Green River Shale (GRS) kerogen in gold capsules for 2–2400 h at 300°C to assess the maturation behaviour of several compounds used as life tracers and for the reconstruction of paleoenvironments (*n*-alkanes, pristane, phytane, gammacerane, steranes, hopanes and cheilanthanes). Lignite samples were maturated in parallel with the GRS kerogen to obtain exact vitrinite reflectance data at every sampling point. Our experiment confirms the applicability of biomarker-based indices and ratios as maturity indicators (e.g. total cheilanthanes/hopanes ratio; sterane and hopane isomerization indices). However, several biomarker ratios that are commonly used for paleoreconstructions (e.g. pristane/phytane, pristane/*n*-C_{17}, phytane/*n*-C_{18} and total steranes/hopanes) were considerably affected by differences in the thermal degradation behaviour of the respective compounds. Short-term experiments (48 h) performed at 400°C also revealed that biomarkers >C_{15} (especially steranes and hopanes) and 'biological' chain length preferences for *n*-alkanes are vanished at a vitrinite reflectance between 1.38 and 1.83% R_O. Our data highlight that 'thermal taphonomy' effects have to be carefully considered in the interpretation of biomarkers in ancient rocks and, potentially, extraterrestrial materials.

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Key words: biomarker ratios, closed system pyrolysis, gas chromatography–mass spectrometry, Green River Shale, maturity indicators, vitrinite reflectance, thermal degradation.

Introduction

Lipid biomarkers are molecular fossils that originate from biological precursor compounds (e.g. Peters *et al.* 2005a; Brocks *et al.* 2016). Ever since Treibs (1936) first discovered chlorophyll-derived porphyrins in ca. 230 Ma old Triassic oil shales, biomarkers have been extensively applied for tracing organisms and evolutionary processes through Earth's history and for the reconstruction of past environments. Effective biomarkers should be source-specific, resistant against thermal- and biodegradation, and analysable in natural samples with routine techniques (e.g. Brocks & Summons 2003; Peters *et al.* 2005a). Therefore lipids are particularly important, because they fulfil these essential demands and their hydrocarbon skeletons have a high potential for preservation over geological timescales (e.g. Brocks & Summons 2003; Summons 2014). Given that the record of body fossils shows huge gaps due to taphonomic effects and the fact that many organisms did not produce preservable hard parts, organic biomarkers may provide a complementary record of past ecosystems independent from the body fossil record (e.g. Hallmann *et al.* 2011; Brocks *et al.* 2016). In that respect, biomarkers have become instrumental for the reconstruction of early Life on Earth and, potentially, for the detection of life in the extraterrestrial realm (e.g. Brocks & Summons 2003; Brocks & Pearson 2005; Hallmann *et al.* 2011; Summons 2014; Olcott Marshall & Cestari 2015).

Despite the wide use of biomarkers for paleoreconstructions, however, it is not always clear how exactly they reflect a paleoecosystem. For instance, commonly less than 1% of organic matter produced in a marine environment is introduced into

the sediment due to abiotic oxidation and intensive microbial reprocessing in the water column and on the sea floor by aerobic and anaerobic respiration (e.g. Hedges & Keil 1995; Peters *et al.* 2005a; Middelburg & Meysman 2007; Vandenbroucke & Largeau 2007). Furthermore, microbially-driven processes observed in microbial mat settings may also profoundly affect the biomarker inventory (i.e. the 'mat seal effect'; Pawlowska *et al.* 2013; Blumenberg *et al.* 2015). During later stages biomarkers are additionally influenced by increasing temperatures (T) and pressures (P). While biomarkers undergo major structural changes during catagenesis (corresponding to temperatures of 50–150°C; Tissot & Welte 1984; Brocks & Summons 2003; Peters *et al.* 2005a), they are almost entirely decomposed during metagenesis and metamorphism (corresponding to temperatures of 150–200°C and >200°C, respectively; Tissot & Welte 1984). High fluid and/or gas pressures, in contrast, may retard the thermal destruction of organic matter (Price 1993). For the decomposition of biomarkers, geological time is commonly considered as an essential factor in addition to temperature (Tissot & Welte 1984; Peters *et al.* 2005b); however, the effect of geological time as a major controlling parameter has also been questioned (Price 1983, 1993). Further geological factors that influence the preservation of biomarkers are the heating rate and mineralogy, especially the adsorption of carbon compounds onto clay mineral surfaces and mineral catalysts that promote the degradation of hydrocarbons (e.g. Mango 1996; Mango & Hightower 1997; Kennedy *et al.* 2002; Brocks & Summons 2003). While many studies focused on the generation, expulsion and migration of petroleum hydrocarbons, it is as yet often poorly constrained as to which extent individual organic compounds are affected by these processes, and whether changing P/T conditions cause shifts in relative abundances and ratios of selected biomarkers.

The main portion of organic matter in sediments and rocks is present in the form of 'kerogen', i.e. macromolecules insoluble in usual organic solvents, acids and bases (as opposed to the solvent-soluble 'bitumen' fraction; Durand 1980). Based on estimations, about 10^{16} tonnes of C are bound to kerogen, compared with only 10^{12} tonnes of living biomass (Durand 1980; Vandenbroucke & Largeau 2007). Kerogen formation is still not fully understood, but essentially includes selective preservation of biomacromolecules and polymerization (especially polycondensation) and immobilization processes of organic molecules during early diagenesis (e.g. Durand 1980; De Leeuw *et al.* 2006; Vandenbroucke & Largeau 2007; Hallmann *et al.* 2011). As macromolecular-bound lipid moieties are more resistant against microbial degradation and thermal overprint than the free molecules, kerogen may facilitate the preservation of biomarkers (e.g. Love *et al.* 1995; Killops & Killops 2005; Hallmann *et al.* 2011). Due to their solid state nature, kerogen particles may remain immobile in the sedimentary environment over geological time scales, and thus are considered syngenetic to the host rock (e.g. Brocks *et al.* 2003a, b; Love *et al.* 2008). Kerogen bound biomarkers are therefore commonly used in the reconstruction of Precambrian ecosystems (e.g. Love *et al.* 2009; Duda *et al.* 2014; Brocks *et al.* 2016). However, it is still poorly known how P and T affect the biomarkers that are bound into the kerogen. Therefore maturation experiments could help to better understand these processes.

Numerous earlier maturation experiments have been conducted on the Green River Shale (GRS) (e.g. Burnham & Singleton 1983; Evans & Felbeck 1983; Huizinga *et al.* 1988; Ruble *et al.* 2001). The GRS comprises relatively immature oil source rocks that contain most lipid biomarker classes regularly used for paleoreconstructions (e.g. Burnham *et al.* 1982; Evans & Felbeck 1983). Not surprisingly, biomarkers in the GRS bitumen have been extensively investigated (e.g. Eglinton & Douglas 1988; Collister *et al.* 1992; Schoell *et al.* 1994; Koopmans *et al.* 1997; Ruble *et al.* 2001), also as possible analogue studies for current and future Mars missions (Olcott Marshall & Cestari 2015). The natural bitumen (i.e. geologically produced) has been reported to contain the major portion of the total steranes and hopanes in the GRS. Another minor portion can be released from kerogen via pyrolysis, showing somewhat different distributions of single steranes and hopanes (Eglinton & Douglas 1988). However, maturation experiments have been mostly focused on petroleum generation from the host rock and changes in the bulk kerogen composition, but so far surprisingly few comprehensive studies exist on the fate of distinct kerogen-bound biomarkers (e.g. Eglinton & Douglas 1988; Monthioux & Landais 1989; Peters *et al.* 1990; Price 1993).

This study aims at assessing the behaviour of selected kerogen-bound hydrocarbon biomarkers, namely *n*-alkanes, pristane, phytane, gammacerane, steranes, hopanes and cheilanthanes, whose presence and ratios are commonly used as life tracers and for the reconstruction of paleoenvironments and evolutionary processes (Peters *et al.* 2005a, b). By conducting hydrous pyrolysis of the GRS kerogen in closed gold capsules at elevated pressures and for different time intervals and temperature regimes, we evaluated the stability of selected compounds, or compound classes, in relation to each other. Our findings reveal major differences in the preservation of biomarkers along the maturation pathway. This 'thermal taphonomy' has potentially wide implications for their interpretation in ancient rocks and, potentially, extraterrestrial materials.

Materials and methods

Samples and maturation experiments

The material used for experimental maturation was an oil shale from the Eocene Green River Formation in Eastern Utah (White River Mine located within BLM Oil Shale Research, Development, and Demonstration Lease UTU-84087). Bituminous brown coal samples (SM20; Gruber & Sachsenhofer 2001) from the Noric Depression (Eastern Alps) were maturated in parallel to the GRS samples (same autoclave, separate gold capsules), to obtain exact vitrinite reflectance values for each maturation step.

The ground GRS rock sample was 3*x* extracted with dichloromethane (DCM). The hydrocarbon fraction prepared from

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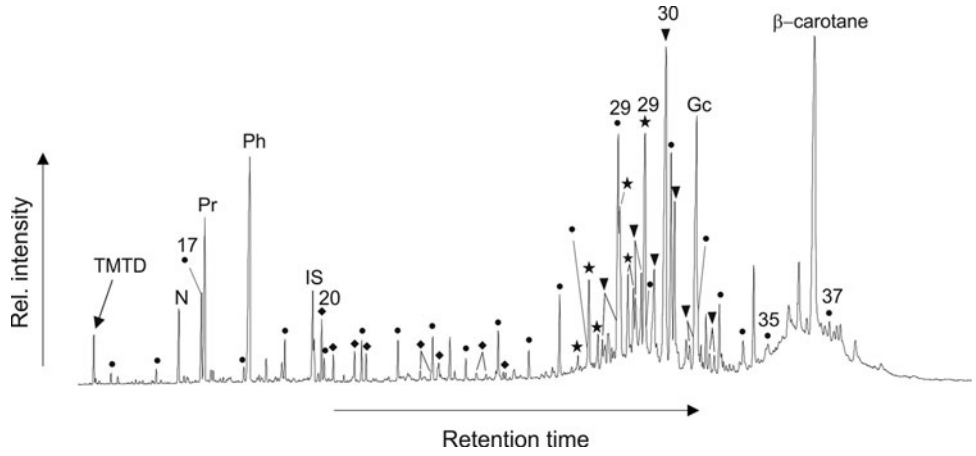

Fig. 1. GC–MS chromatogram (total ion current, TIC) of Green River Shale bitumen (untreated). Numbers refer to number of carbon atoms. Filled circles denote *n*-alkanes, stars denote steranes, triangles denote hopanes, diamonds denote cheilanthanes; TMTD,2,6,10 trimethyl-tridecane; N, norpristane; Pr, pristane; Ph, phytane; Gc, gammacerane; IS, internal standard (eicosane d42 10 mg l^{-1}).

the resulting total extract (see the section 'Extraction and fractionation') was used as the untreated reference (0 h). Kerogen was isolated from the pre-extracted GRS using hydrochloric acid and hydrofluoric acid (see e.g. review by Durand & Nicaise 1980). The residue was homogenised with a mortar and again 3*x* extracted with DCM to remove residual bitumen. The dried kerogen (ca. 10 mg) was sealed into gold capsules (3.0 mm outer diameter, 2.6 mm inner diameter, 15 mm length) by arc welding using a Lampert™ tungsten inert gas impulse micro welding device. Extensive heating of the sample during welding was avoided by wrapping the capsule in wet tissue paper. Samples were matured at 300°C and 2 kbar for 2 to 2400 h (100 days). Four additional short duration experiments at 400°C (2, 5, 24 and 48 h) were performed to check for the upper limit of biomarker preservation. Experiments with 2–720 h durations were carried out in rapid-quench cold seal pressure vessels (CSPV) enabling a fast heating and cooling of the samples within a few seconds (see Schmidt *et al.* 2013 for technical details). The 2400 h duration experiments were conducted in conventional CSPV with slower heating and cooling rates (see Le Bayon *et al.* 2012 for technical details).

Extraction and fractionation

After maturation, the capsules were opened using a metal spike, forceps and scissors, which were thoroughly cleaned between the samples using acetone. About 2/3 of the material was then extracted 4*x* with each 2 ml of an *n*-hexane/DCM mixture (1:1, V:V; 20 min ultrasonication). The resulting extracts were combined and carefully reduced using N_2. The extracts were never completely dried before the next step of processing to avoid a major loss of low-boiling compounds (Ahmed & George 2004). Extracts were dried onto a small amount of silica gel and fractionated by column chromatography (diameter: 1.5 cm, height: 8 cm, Merck silica gel 60). Saturated hydrocarbons were eluted with 3 ml *n*-hexane. The resulting saturated hydrocarbon fractions were reduced to 1 ml and analysed by gas chromatography–mass spectrometry (GC–MS).

GC–MS and vitrinite reflectance

GC-MS analyses were carried out using a Thermo Fisher Trace 1300 Series GC coupled to a Thermo Fisher Quantum XLS Ultra MS. The GC was equipped with a capillary column (Phenomenex Zebron ZB-1MS/Phenomenex Zebron ZB-5, 30 m, 0.1 µm film thickness, inner diameter 0.25 mm). Fractions were injected into a splitless injector and transferred to the GC column at 270°C. The carrier gas was He with a flow rate of 1.5 ml min^{-1}. The GC oven temperature was ramped 80°C (1 min) to 310°C at 5°C min^{-1} (held 20 min). Electron ionization mass spectra were recorded in full scan mode at 70 eV electron energy with a mass range of *m*/*z* 50–600 and a scan time of 0.42 s.

To determine vitrinite reflectance of the reference coal samples, samples were embedded into epoxy and afterwards cut, abraded (four steps; P400 to P2500), and polished (diamond and alumina slurries, four steps, grain sizes 9–0.05 µm, each step 5 min). After polishing the minimum and maximum reflectance of vitrinites have been measured in linear polarised light at 546 nm using a SpectraVision PMT system (A.S. & Co.) consisting of a Zeiss Axio Imager.A2m microscope (Carl Zeiss Microscopy, LLC, New York, United States) with an attached Zeiss MCS CCD/UVNIR spectrometer. Fifteen measurements on random grains were made for each sample. Standard deviations were generally <0.08%.

Results and discussion

The hydrocarbon fraction of the untreated GRS bitumen (0 h) contains *n*-alkanes, acyclic (e.g. pristane and phytane) and cyclic (e.g. steranes and terpanes) isoprenoids as well as some other biomarkers that are not in the focus of this study (e.g. carotenoids; Fig. 1). The 300°C experiment covers a maturity range from immature (0.49% R_O, untreated sample 0 h) to the main catagenetic stage (1.21% R_O, 2400 h) thus being well suited to track continuous changes in the biomarker inventory with increasing maturity. The 400°C experiment conducted to check for the upper limit of biomarker preservation

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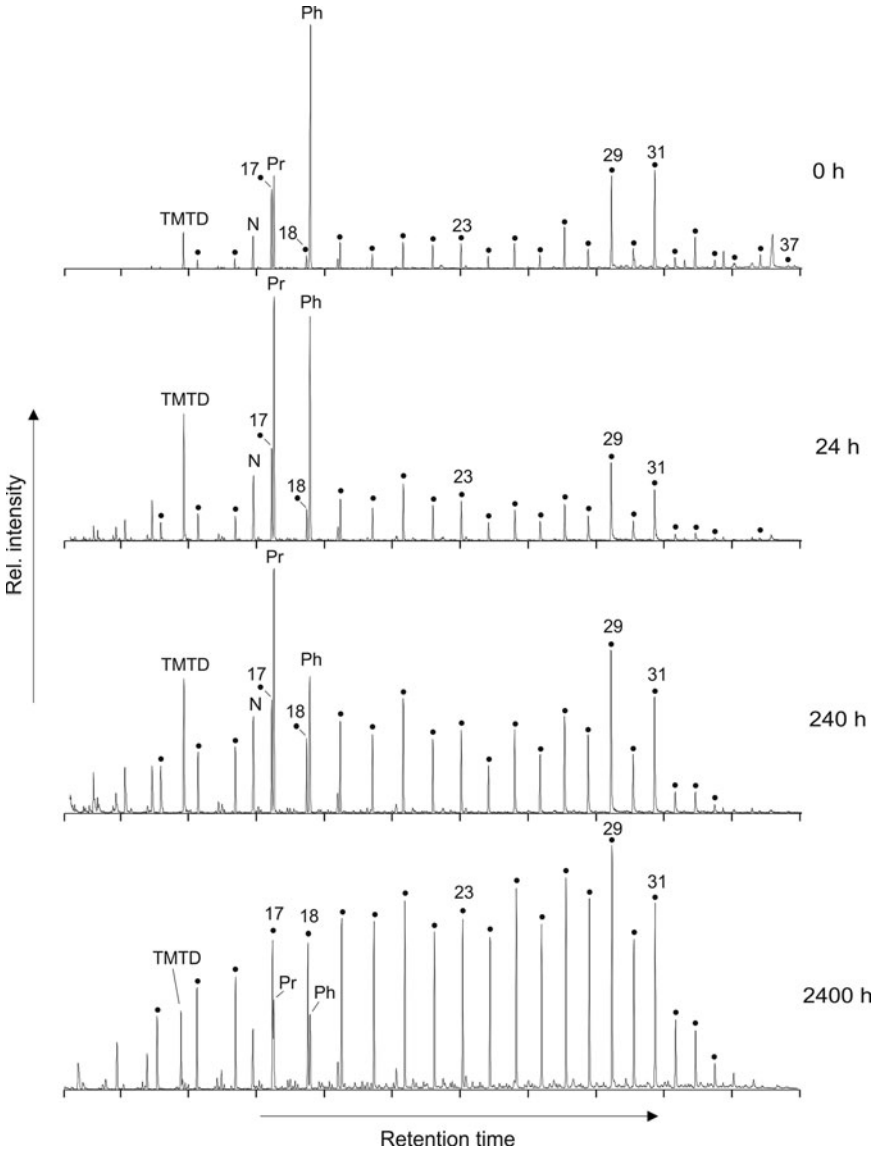

Fig. 2. GC–MS chromatograms (*n*-alkanes and isoprenoids, *m*/*z* 85) of Green River Shale bitumen (untreated, 0 h) and experimentally maturated Green River Shale kerogens (300°C; 24, 240 and 2400 h, respectively). Numbers refer to number of carbon atoms; filled circles denote *n*-alkanes; TMTD, 2,6,10 trimethyl-tridecane; N, norpristane; Pr, pristane; Ph, phytane.

represents a maturity range between 1.19% R_O (2 h) and 1.83% R_O (48 h).

n-Alkanes

n-Alkanes in the original GRS bitumen have chain lengths from *n*-C_{15} to *n*-C_{37}. They show a bimodal distribution with maxima at *n*-C_{17}, and *n*-C_{29}/*n*-C_{31} (Fig. 2). In the long-chain range, *n*-alkanes exhibit a clear odd-over-even predominance as reflected by a carbon preference index (CPI) of 3.6 (calculated after Bray & Evans 1961; Table 1). *n*-C_{17} is common in extant cyanobacteria and algae and its predominance in the short-chain range can be attributed to planktonic photoautotrophs (e.g. Blumer *et al.* 1971; Hoffmann *et al.* 1987; Jacobson *et al.* 1988). The also abundant long chain *n*-alkanes with a pronounced odd-over-even predominance in the range of *n*-C_{27} to *n*-C_{33} are commonly explained by inputs of epicuticular leaf waxes derived from higher land plants (Eglinton & Hamilton 1967). The *n*-alkane distribution found in the GRS bitumen is thus well in line with a high-productivity lacustrine environment (e.g. Tissot *et al.* 1978; Horsfield *et al.* 1994). During experimental maturation, this original *n*-alkane distribution is profoundly altered. While the distinct maximum at *n*-C_{17} and the odd-over-even predominance in the high molecular weight range become less pronounced (Fig. 2, 24–2400 h), the CPI decreases from 3.6 (0 h) to 1.4 after 2400 h (1.21% R_O, Table 1). It should be noted that these experimental data reflect a lag of the CPI with respect to vitrinite reflectance, as a CPI-value approaching 1 is generally expected at ∼0.9% R_O (i.e. peak oil window; Peters *et al.* 2005b). In our experiment, the 'biological' chain length preferences of *n*-alkanes are completely vanished only after 48 h at 400°C (corresponding to 1.83% R_O, not shown). Possible

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Table 1. *Biomarker ratios, indices and corresponding vitrinite reflectances (VR) of Green River Shale bitumen and experimentally maturated kerogens* (300°C)

Maturation time (hours)	0	2	5	10	24	48	120	240	360	720	2400
CPI	3.6	2.6	2.8	2.7	2.6	2.6	2.2	2.0	1.8	1.7	1.4
VR (% R_O) mean	0.49	0.69	0.64	0.73	0.79	0.85	0.89	0.92	0.96	1.03	1.21
VR (% R_O) SD	0.03	0.05	0.03	0.05	0.04	0.04	0.02	0.06	0.03	0.04	0.04
Pristane/phytane	0.3	0.4	0.5	0.7	1.1	1.2	1.5	1.7	1.6	1.5	1.1
Pristane/*n*-C17	1.4	1.8	2.0	2.8	3.5	4.8	3.6	2.9	2.7	1.4	0.9
Phytane/*n*-C18	26.5	28.0	21.0	13.7	9.9	9.3	4.4	2.6	2.4	1.2	0.8
Total steranes/hopanes	0.4	0.3	0.3	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1
Total cheilanthanes/hopanes	0.1	0.2	0.2	0.2	0.3	0.3	0.4	0.4	0.4	0.4	1.0
C_{29} 20S/(20S + 20R)	0.23	0.23	0.23	0.23	0.27	0.29	0.38	0.41	0.48	0.53	0.59
C_{31} 22S/(22S + 22R)	0.37	0.37	0.38	0.41	0.45	0.52	0.55	0.56	0.60	0.56	0.55
C_{32} 22S/(22S + 22R)	0.45	0.22	0.26	0.31	0.35	0.43	0.48	0.56	0.57	0.58	0.58
Gammacerane index	4.5	4.1	4.2	4.3	4.2	4.5	4.5	4.6	4.3	4.8	5.3

All ratios and indices were calculated from peak integrals: Isoprenoids and *n*-alkanes, m/z 85; steranes, m/z 217; hopanes and cheilanthanes, m/z 191. CPI, carbon preference index (after Bray & Evans 1961); SD, standard deviation ($n = 15$); Gammacerane index = 10 × gammacerane/(gammacerane + C_{30} hopane).

reasons for this mismatch between biomarkers and vitrinite reflectance are discussed below. Nevertheless, our results underline that the source-specific features of individual *n*-alkane chain lengths gradually disappear with increasing maturity (Tissot *et al.* 1978; Price 1993; Peters *et al.* 2005a).

Acyclic isoprenoids

Acyclic isoprenoids in the GRS bitumen include 2,6,10-trimethyltridecane (C_{16}), norpristane (C_{18}), pristane (C_{19}) and phytane (C_{20}) (Figs 1 and 2). The pristane/phytane ratio (Pr/Ph) is 0.3, while the pristane/*n*-C_{17} (Pr/*n*-C_{17}) and phytane/*n*-C_{18} (Ph/*n*-C_{18}) ratios are 1.4 and 26.5, respectively (Table 1). Pristane and phytane are biomarkers that mainly originate from phytol, the corresponding alcohol to the phytyl side chain of chlorophyll (Brocks & Summons 2003; Peters *et al.* 2005b). However, pristane can also derive from tocopherols (E vitamins) (Goossens *et al.* 1984), while phytane can derive from archaeols (i.e. ether lipid compounds from Archaea; Brocks & Summons 2003). Pristane in oils and bitumens can furthermore originate from cracking of isoprenoid moieties that are bound to the kerogen matrix (Larter *et al.* 1979; Goossens *et al.* 1988a, b). However, given the type of environment (see above), chlorophyll and/or tocopherols are likely to be the major precursors for these isoprenoids in the GRS.

Pr/Ph ratios are commonly used for the reconstruction of paleoredox conditions in ancient settings (e.g. Blumenberg *et al.* 2012; Yamada *et al.* 2014; Tulipani *et al.* 2015; Luo *et al.* 2015; Naeher & Grice 2015), as phytol is preferentially transformed to pristane under oxic conditions and to phytane under anoxic conditions (Didyk *et al.* 1978; Peters *et al.* 2005b). However, the Pr/Ph ratio is also affected by thermal maturity (Ten Haven *et al.* 1987; Peters *et al.* 2005b). Our data show that the Pr/Ph ratio increases until a maturation time of 240 h, but then decreases (Fig. 3(a)). This change in the evolution of Pr/Ph ratios could be due to a preferential release of pristane from kerogen during early catagenesis (Peters *et al.* 2005b).

The Pr/*n*-C_{17} and Ph/*n*-C_{18} ratios are used to assess the impact of biodegradation and thermal maturation on bitumens (e.g. Hieshima & Pratt 1991; Meredith *et al.* 2000; Peters *et al.* 2005a, b; Blumenberg *et al.* 2012). Both ratios typically increase due to biodegradation (Peters *et al.* 2005a, b) and sdecrease with increasing maturity (Alexander *et al.* 1981; Ten Haven *et al.* 1987; Peters *et al.* 2005b). As expected, our data also show a decrease in Ph/*n*-C_{18} over time, albeit with a somewhat steeper drop of the ratio before 100 h (Fig. 3(b)). This could be explained by a time lag between the formation of phytane and *n*-C_{18} from kerogen; phytane is dominantly generated during earliest maturation whereas a pronounced formation of *n*-C_{18} occurs at higher maturities. This effect may be influenced by heating rate (Burnham *et al.* 1982) or kerogen composition.

The impact of maturity on Pr/*n*-C_{17} seems to be more complex as the ratio initially increases, summits at 48 h and then decreases with further maturation time (Fig. 3(b)). In natural samples, an increase of the Pr/*n*-C_{17} ratio may result from preferential biodegradation of the *n*-alkane (Peters *et al.* 2005b), which can be excluded here. Instead, the observed phenomenon may be explained by a higher release of pristane relative to *n*-C_{17} from the kerogen during early maturation (e.g. from tocopherol or other isoprenoid moieties; Larter *et al.* 1979; Goossens *et al.* 1984; Goossens *et al.* 1988b). This is in line with the observed initial increase of the Pr/Ph ratio (Fig. 3 (a)). Taken together our data suggest a more reliable applicability of Ph/*n*-C_{18} as a maturity indicator, while that of Pr/*n*-C_{17} is restricted.

Cyclic isoprenoids

Cyclic isoprenoids in the GRS bitumen include steranes, hopanes, gammacerane and cheilanthanes (Fig. 1). The 20S and 20R isomers of C_{27} to C_{29} steranes are abundant (Figs 1 and 4). Hopanes range from C_{27} to C_{32}, with the C_{30} pseudohomologue being particularly abundant (Figs 1 and 4). For all homohopanes (i.e. hopanes >C_{30}), both 22S and 22R isomers are

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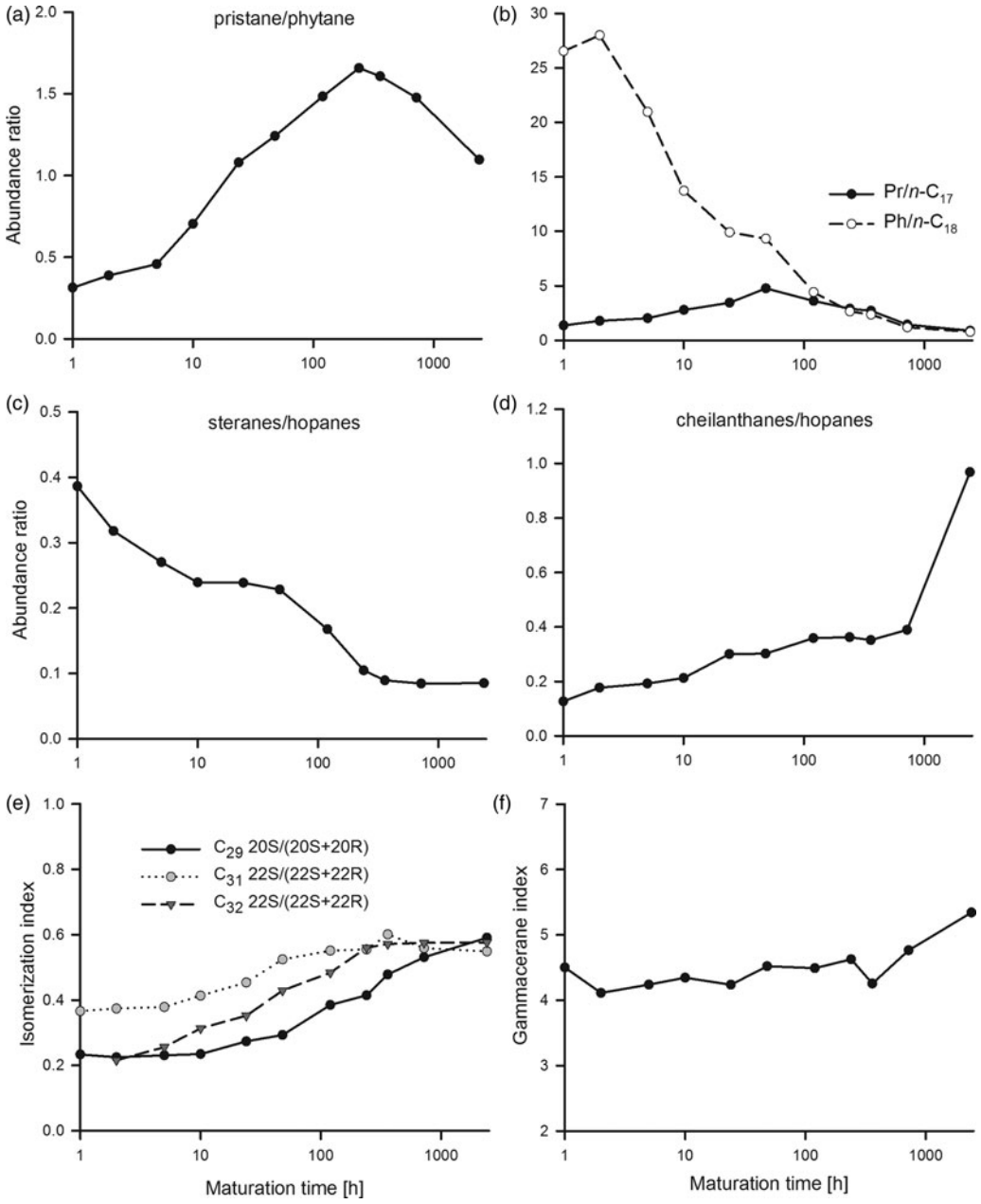

Fig. 3. Evolution of biomarker-based ratios and indices of untreated (0 h) and experimentally maturated (2–2400 h) Green River Shale samples (see also Table 1). Please note that the untreated Green River Shale bitumen is always plotted at 1 h (instead of 0 h) due to the logarithmic scale. Gammacerane index = 10 × gammacerane/(gammacerane + C_{30} hopane). Pr, pristane; Ph, phytane.

present (Fig. 4). Cheilanthanes range from C_{20} to C_{26} (Fig. 4). All these compounds are useful biosignatures. While steranes derive from sterols, which control the permeability and rigidity of eukaryotic cell membranes (e.g. Brocks & Summons 2003; Peters *et al.* 2005b), hopanes mainly originate from bacteriohopanepolyols (C_{35}), which are lipids in some groups of bacteria (Rohmer *et al.* 1984; Brocks & Pearson 2005). The pentacyclic triterpenoid gammacerane (C_{30}) derives from tetrahymanol (Ten Haven *et al.* 1989), which has various biological sources but is particularly abundant in anoxygenic phototrophs (*Rhodopseudomonas palustris*; Kleemann *et al.* 1990; Eickhoff *et al.* 2013) and bacterivorous ciliates (Harvey & McManus 1991; Sinninghe Damsté *et al.* 1995). Cheilanthanes are tricyclic terpanes (usually C_{19} to C_{45}, rarely up to C_{54}) that were applied as biomarkers in many studies (Moldowan *et al.* 1983; De Grande *et al.* 1993; Brocks & Summons 2003; Brocks & Pearson 2005; Peters *et al.* 2005b, and references therein). The actual source of cheilanthanes is still unknown but it has been suggested that the precursor lipids originate from eukaryotic algae (e.g. Brocks & Summons 2003; Brocks & Pearson 2005).

The total steranes/hopanes ratio (St/H) is commonly used to evaluate relative inputs of eukaryotic and prokaryotic biomass (e.g. Brocks *et al.* 1999; Peters *et al.* 2005b; Love *et al.* 2009;

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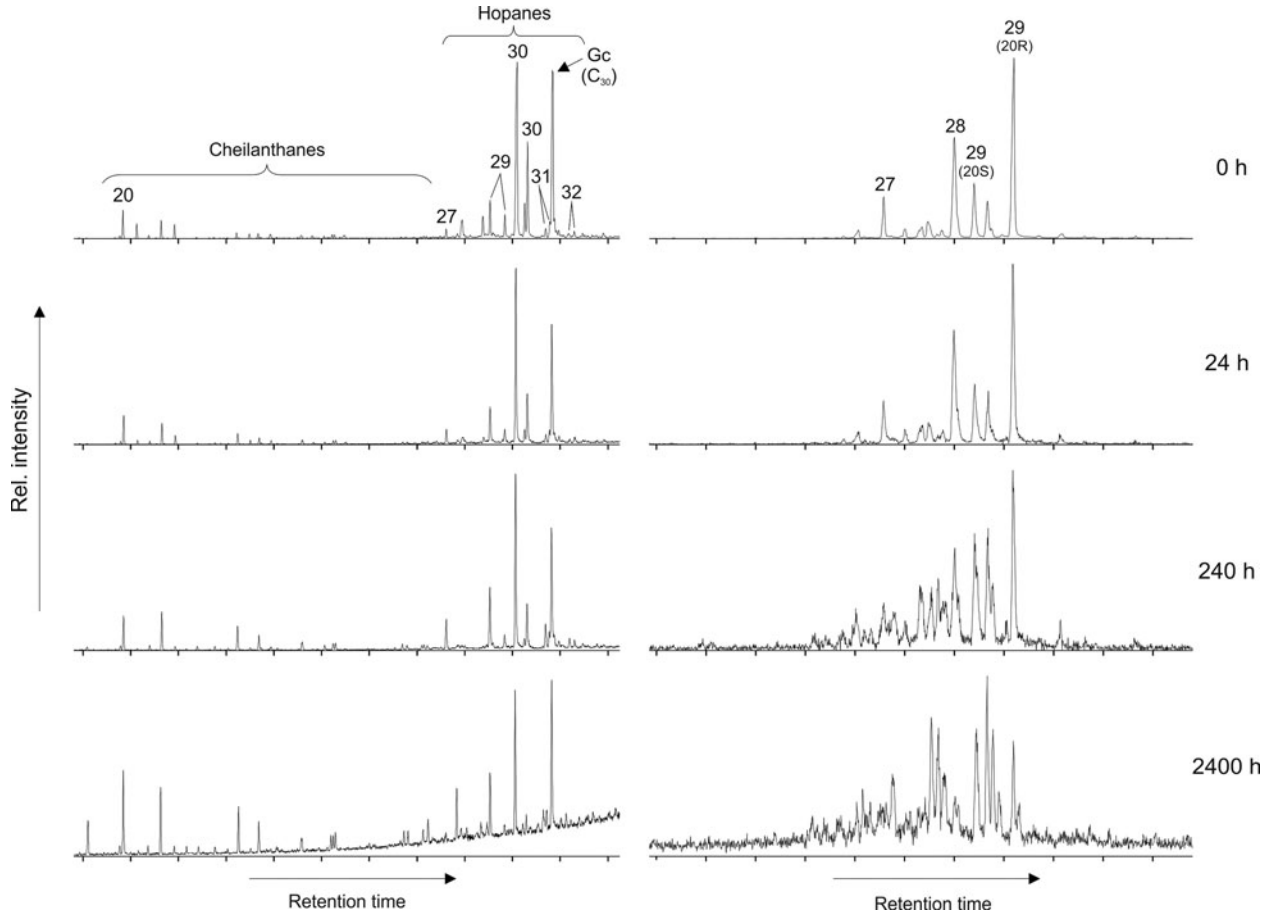

Fig. 4. GC–MS chromatograms of terpanes (*m/z* 191, left side) and steranes (*m/z* 217, right side) in the Green River Shale bitumen (untreated, 0 h) and experimentally maturated Green River Shale kerogens (300°C; 24, 240 and 2400 h, respectively). Numbers refer to number of carbon atoms. Gc, gammacerane.

Blumenberg *et al.* 2012; Flannery & George 2014). However, it is known that this ratio may change through taphonomic processes (Pawlowska *et al.* 2013; Blumenberg *et al.* 2015). Also thermal maturation appears to be critical, leading to a decreasing St/H ratio (Requejo 1994; Norgate *et al.* 1999). In our 300°C experiment, the St/H ratio shows a four-fold decrease from 0.4 to 0.1, testifying a strong impact of maturity in the range of 0.49–1.21% R_O (Table 1, Fig. 3(c)). At even higher maturities, in the 400°C experiment, a point was reached where steranes were entirely decomposed, whereas hopanes were still present (St/H = 0 at 1.38% R_O). This different behaviour of steranes and hopanes has to be considered in addition to taphonomic processes in paleoenvironmental studies, particularly in ancient rocks of higher maturities.

Further biomarker based maturity proxies include the total cheilanthanes/hopanes ratio (Chei/H) as well as isomerization indices of homohopanes (22S/(22S + 22R)) and steranes (20S/(20S + 20R)) (e.g. Rullkötter *et al.* 1986; Summons *et al.* 1988; Brocks *et al.* 1999, 2016; Ruble *et al.* 2001; Peters *et al.* 2005b; Blumenberg *et al.* 2012). Chei/H depends on maturity as cheilanthanes are more resistant to thermal maturation than hopanes and are preferentially released from the kerogen with increasing maturity (Aquino Neto *et al.* 1981; Peters *et al.* 1990). In our 300°C experiment, this is confirmed by a continuous increase of the Chei/H ratio from 0.1 to 1.0 (Fig. 3(d), Table 1). In the case of homohopane and sterane isomerization, the biological R-configuration is progressively transferred to the S-configuration during maturation, thus leading to a relative increase of the S isomers (Brocks & Summons 2003; Peters *et al.* 2005b; Hallmann *et al.* 2011). In our experiment, sterane and hopane isomerization consistently increased with maturation time, as expected (Fig. 3(e)). Thermal equilibrium values (22S/(22S + 22R) hopanes: 0.57–0.62, 20S/(20S + 20R) steranes: 0.52–0.55; Peters *et al.* 2005b) were reached after 360 h (hopanes) and 720 h (steranes), which in our experiments correspond to 0.96 and 1.03% R_O, respectively (Table 1). Thus, a shift can be seen between our experimental data and published biomarker equilibrium values, as full isomerization in geological systems is expected at roughly 0.6 and 0.8% R_O, respectively (Peters *et al.* 2005b). This mismatch may, to some degree, be due to differences in the maturation behaviour between the two samples (lignite versus GRS), as well as a lack of resolution due to the setting of time intervals for the experiments. On the other hand, it is commonly observed that vitrinite reflectance data and biomarkers may give different results regarding thermal maturity, with variations of +/−0.1% R_O being common (Peters *et al.* 2005b). Although both vitrinite reflectance and biomarker indicators

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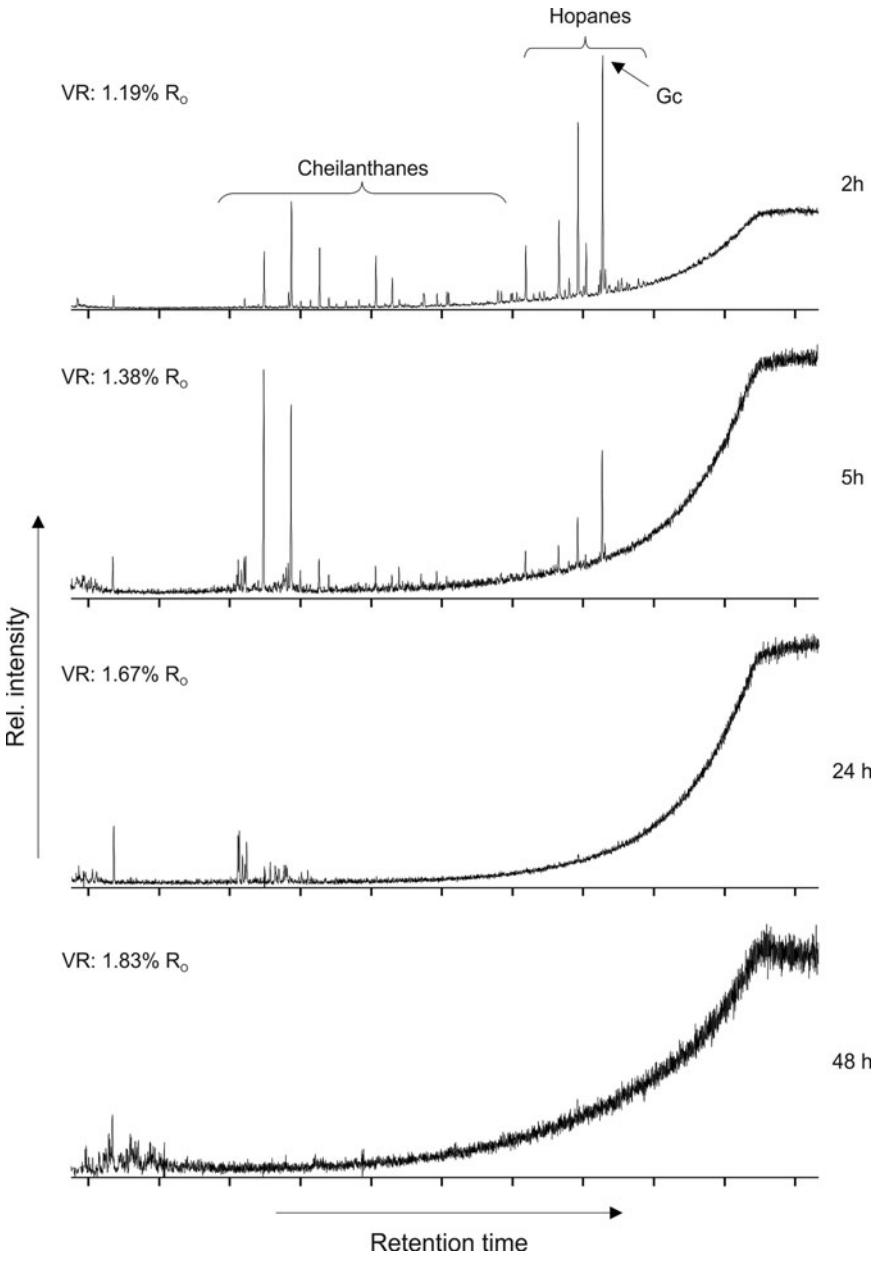

Fig. 5. GC–MS chromatograms (terpanes, *m/z* 191) of experimentally maturated Green River Shale kerogens (400°C; 2, 5, 24 and 48 h) with corresponding vitrinite reflectance (VR). Gc, Gammacerane.

are controlled by thermal stress (temperature and time), biomarkers may be additionally influenced by other effects. For example, the CPI is also affected by organic matter input (source effect) and biodegradation, while isomerization indices might be influenced by selective thermal decomposition of individual isomers, and/or fast reverse reactions (see Peters *et al.* 2005b for detailed discussion). Therefore the comparability of short-term experimental data to natural samples is limited.

The pronounced occurrence of gammacerane and a high gammacerane index (i.e. $10 \times Gc/(Gc + C_{30}$ hopane)) are highly specific indicators for water column stratification and, potentially, increased salinity (Sinninghe Damsté *et al.* 1995; Peters *et al.* 2005b). Our data show that the gammacerane index varies little in the course of the 300°C experiment (Fig. 3(f)), underlining that this proxy is barely affected by maturation processes.

When are the biomarkers gone?

The 400°C experiment (1.19–1.83% R_O) provides information on the overall thermal stability of biomarkers beyond the diagenetic/catagenetic stages. Among the biomarkers investigated in this study, steranes turned out as the thermally least stable compounds. While traces of steranes were still present at the end of the 300°C experiment (1.19% R_O, Fig. 4), these molecules were completely destroyed after 5 h at 400°C, corresponding to 1.38% R_O (Fig. 5). In contrast, hopanes,

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cheilanthanes and gammacerane were shown to be thermally more stable (in accordance with e.g. Eglinton & Douglas 1988). In our experiment, traces were still present after 24 h at 400°C (1.67% R_O), while these compounds were no longer detected after 48 h, corresponding to 1.83% R_O (Fig. 5).

It has been postulated that in the range of 1.35–2.0% R_O all biomarkers >C_{15} are destroyed (Tissot & Welte 1984), which is in good accordance with our results. As this range represents the end of catagenesis (~150°C in natural systems), the preservation of biomarkers in Precambrian rocks that experienced extensive metamorphism should be the exception (see e.g. French *et al.* 2015). Yet, it has been shown that sedimentary rocks may still contain hydrocarbons (and biomarkers) >C_{15} even if they experienced burial metamorphism (i.e. >200°C, >4% R_O; e.g. Price 1993; Brocks & Summons 2003, and references therein). This implies that the preservation of biomarkers in natural systems is also influenced by further parameters such as lithology (grain size), reaction kinetics, presence of water, and fluid pressures (Price 1993). This stresses the need for further maturation experiments in which these parameters are considered.

Conclusions

Our experimental maturation performed on kerogen from the Eocene Green River Shale study confirms the applicability of biomarker-based indices and ratios as maturity indicators (e.g. total cheilanthanes/hopanes ratio; sterane and hopane isomerization indices). However, the data also demonstrate that several biomarker ratios that are commonly used for paleobiological and environmental interpretations (e.g. pristane/phytane, pristane/*n*-C_{17}, phytane/*n*-C_{18} and total steranes/hopanes) are profoundly biased by differences in the thermal degradation behaviour and therefore have to be used only upon consideration of the thermal history of the sample. In particular, a strong relative increase of pristane versus phytane during the early maturation stages indicates different sources and mechanisms of formation for these compounds and gives rise to major shifts in the pristane/*n*-C_{17} and phytane/*n*-C_{18} ratios. Likewise, the ratio of eukaryote-derived steranes versus bacterial-derived hopanes strongly decreases in the course of maturation, indicating that steranes are considerably more susceptible to thermal degradation than hopanes. On the basis of the presence or relative abundance of steranes (total steranes/hopanes ratio), organic matter contributions of eukaryotes to ancient sediments may therefore be underestimated. On the other hand, bacterial hopanes and gammacerane (derived from anoxygenic bacteria and/or bacterivorous ciliates) showed very similar stability, so that the gammacerane index remained virtually unaffected by thermal maturation. In our experiment, biomarkers >C_{15} (especially steranes and hopanes) and 'biological' chain length preferences for *n*-alkanes were completely vanished at 1.83% R_O. However, a systematic shift was observed between measured vitrinite reflectances and molecular maturity indicators, resulting in R_O values consistently higher than expected for a given biomarker ratio. Summarising, our study nevertheless underlines the value of maturation experiments for assessing the influence of thermal stress on individual biomarkers, or biomarker ratios. More such experiments have to be performed to improve our ability of assessing the impact of maturation on organic matter in ancient rocks and, potentially, extraterrestrial materials. Future studies should also employ different P/T regimes and matrix minerals for a better estimation of their impact on the preservation of individual biomarkers.

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