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Possible algal origin of long chain odd *n*-alkanes in immature sediments as revealed by distributions and carbon isotope ratios

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Abstract- A Pliocene oil shale (Pula, Hungary), a C_3 plant *Triticum aestivum* and a C_4 plant *Zea mays* were compared using isotopic composition of bulk organic matter, along with distributions and individual carbon isotope ratios of *n*-alkanes from organic extracts. The microalga *Botryococcus braunii* (A race) was thus shown to be the main source of the predominant 27, 29 and 31 *n*-alkanes of Pula sediment. Therefore, the dominance of odd carbon-numbered *n*-alkanes in the range C_{25} to C_{35} in extracts from immature sediments shall not be systematically assigned to higher plant contribution but algal input has also to be tested *via* molecular isotope ratios. In fact, the long chain *n*-alkanes with an odd predominance observed in extracts of various immature sediments are likely to be derived, at least partially, from algae.

Key words - carbon-13, *n*-alkane, *n*-alkene, Pula oil shale, *Botryococcus braunii,* alga, plant, waxes, sediment.

INTRODUCTION

n-Alkanes occur almost ubiquitously in soils, sediments, petroleums and coals (Eglinton, 1969; Morrisson, 1969; Albrecht and Ourisson, 1971; Tissot and Welte, 1984) and their distribution has been widely used for source organism identification and as a maturation parameter. Markedly different pathways can be implicated, however, in *n*-alkane formation. Indeed, such compounds are likely to represent a direct contribution of natural waxes in some immature sediments, whereas they appear to be mainly formed by thermal breakdown of kerogen in mature sediments and petroleums. In this connection, kerogen fractions derived from the selective preservation of resistant, highly aliphatic, biomacromolecules have been recently shown to be especially prolific materials for the catagenic production of *n*-alkanes (Largeau *et al*., 1986; Tegelaar *et al*., 1989; De Leeuw and Largeau, 1993). *n*-Alkanes can also arise by degradation of biological aliphatic precursors such as *n*-alcohols and *n*-carboxylic acids (Tissot and Welte, 1984; Lichtfouse and Collister, 1992; and refs. therein).

n-Alkanes exhibiting a pronounced odd carbon-number predominance in the range C_{25} to C_{35} have often been used to indicate a terrestrial contribution in sediments because similar distributions are observed in leaf waxes of higher plants. However, *n*-alkenes also showing an odd predominance in the same range occur in various microalgae (Kolattukudy, 1976). Through diagenetic reduction, these algal constituents might thus represent an important source of sedimentary *n*-alkanes. Isotopic analysis at the molecular level is actually the sole method which could allow to discriminate between these two origins and thus provide direct

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information on contributing organisms. In the present study, this method was applied to the hydrocarbons isolated from the extract of an important, organic-rich, Pliocene deposit from Pula (Hungary). The bulk carbon isotope ratio of this oil shale was also determined. Parallel measurements were carried out, for comparative purposes, on *Triticum aestivum*, a C_3 plant, and *Zea mays*, a C_4 plant. The major aim of these isotopic studies was to specify the origin of the *n*-alkanes occurring in Pula extract, to derive information on the relative contributions of terrestrial and algal inputs to this sediment and, in a more general way, to test the suitability of odd, long chain, *n*-alkanes as markers of higher plant contribution.

EXPERIMENTAL

Detailed procedures are described elsewhere (Metzger *et al*., 1986; Lichtfouse *et al*., 1994). Leaves from two plants of different mode of CO_2 fixation, *T. aestivum* and *Z. mays*, were collected in 1992 in an experimental field at Boigneville, France. The tested Pula sample contained about 45% of TOC; this oil shale, comprising immature type I kerogen, is a typical maar lake deposit (Brukner-Wein *et al*., 1991). *n*-Alkanes were isolated from Pula sediment extract and leaf extracts, then purified by successive column and thin layer chromatography. They were identified by gas chromatography-mass spectrometry and co-elution with standards. Bulk isotopic compositions were measured on a Carlo Erba NA 1500 elemental N and C analyser coupled to a VG Sira 10 mass spectrometer. Such measurements were carried out on *Z. mays* and *T. aestivum* leaves, on HCl-decarbonated Pula oil shale and on the kerogen isolated from this shale by a classical HCl-HF treatment. Isotopic analyses of individual *n*-alkanes were carried out under a continuous helium flow using an HP 5890 gas chromatograph coupled with a CuO furnace (850°C) and a cryogenic trap (-100°C) coupled with a VG Optima mass spectrometer, monitoring continuously ion currents at m/z = 44, 45 and 46. Carbon isotopic compositions are expressed in per mil. relative to the Pee Dee Belemnite standard: $\delta^{13}C = [(^{13}C/^{12}C \text{ sample} - {}^{13}C/^{12}C \text{ std})/(^{13}C/^{12}C \text{ std})] \times 10^3$, where $^{13}C/^{12}C$ std = 0.0112372. Significant measurements of isotope ratios cannot be achieved when some overlapping of GC peaks occurs (Lichtfouse *et al*., 1991). Accordingly, the isotopic composition of *n*-heptacosane from *Pula* sediment is not reported due to the presence of a nearly co-eluting C_{27} monounsaturated hydrocarbon.

RESULTS AND DISCUSSION

Isotopic composition of bulk organic matter

Common higher plants can be classified into two isotopic categories according to their mode of CO_2 fixation (Smith and Epstein, 1971, O'Leary, 1981). C_3 plants incorporate CO_2 from the atmosphere by ribulose bisphosphate carboxylation (Calvin cycle) and show isotopic values ranging from -34‰ to -24‰. C_4 plants fix CO_2 by phosphoenol pyruvate carboxylation (Hatch-Slack cycle) and show isotopic values ranging from -19‰ to -6‰. Algae have intermediate values of -23‰ to -12‰.

To evaluate the contribution of terrestrial plants in Pula sediment, the bulk isotopic compositions of this oil shale, of a modern C_3 plant (wheat) and a modern C_4 plant (maize) were compared. However, such a comparison must take into account the difference of isotopic composition of atmospheric CO_2 between Pliocene and present. The present isotopic composition of CO_2 is 7.8‰ while the pre-industrial value was about 1-1.5‰ heavier and did not undergo significant changes during geological times, except sharp variations at era boundaries (Shackleton, 1987, Schlanger *et al*., 1987, Marino and McElroy, 1991, Raymo *et al*., 1992). Accordingly, a corrective term of +1.5‰ was applied to modern isotopic values in order to allow for a significant comparison with Pula sediment (Table 1).

Table 1. Carbon isotope composition of bulk organic matter and *n*-alkanes from maize leaves, a C_4 plant, wheat leaves, a C_3 plant, and Pula sediment. Values in parentheses take into account the isotopic difference between ancient and modern atmospheric CO_2 (+1.5‰).

	Bulk organic matter	*n*-alkanes (carbon number)							
		27	**28**	**29**	**30**	**31**	**32**	**33**	**34**
Zea Mays (maize)	-13.18	-19.1		-18.4		-20.6		-22.2	
	(-11.68)	(-17.6)		(-16.9)		(-19.1)		(-20.7)	
Triticum aestivum (wheat)	-29.61	-35.9		-36.5		-36.7		-36.7	
	(-28.11)	(-34.4)		(-35.0)		(-35.2)		(-35.2)	
Pula sediment	-19.03*		-28.7	-30.7	-28.1	-30.9	-28.8	-30.0	-28.8

* It is well documented that *B. braunii* fossilization takes place *via* the selective preservation of the non-hydrolysable aliphatic biopolymer building up the outer walls. In the living algae, this material is formed by polymerization of high molecular weight lipids comprising long alkyl chains. In the A race, the biosynthetic pathways implicated in the production of the resistant biopolymer and of *n*-alkadienes are related. Accordingly, relatively close isotopic ratios are expected for the above compounds. The large difference noted between the bulk isotope ratio of the HCl-decarbonated sediment, on one hand, and the *n*-alkanes of sediment extract, on the other hand, shall reflect the somewhat heterogenous nature of the sediment. Indeed, even in Torbanites, which are sedimentary rocks chiefly composed of fossil *Botryococcus* accumulation, micro-FTIR observations revealed the presence of an interstitial organo-mineral matrix which organic constituents are sharply different from those of *Botryococcus* colonies (Landais *et al.*, 1993). In fact, further treatment of the decarbonated Pula sediment with HF-HCl results in a marked shift of the bulk isotope ratio to a lighter value: the kerogen isolated after elimination of the chemically labile constituents exhibits a bulk isotope ratio of -23.68‰. The difference still observed between this value and the average isotope ratios of the extracted *n*-alkanes should mainly reflect the occurrence, along with the predominant *Botryococcus* colonies observed by SEM, of non-hydrolysable materials derived from other sources.

The pronounced isotopic differences between the sediment and the two plants indicate that a large input of terrestrial material is unlikely (Table 1). Furthermore, the isotopic value of the Pliocene sediment (-19.03 ‰) falls into the corrected isotopic range of algae (-21.5‰ to -10.5‰). The major algal contribution thus suggested is consistent with the abundant presence of fossil remains of the colonial microalga *Botryococcus braunii* observed by scanning electron microscopy in Pula oil shale (Solti, 1985).

n-Alkane distributions and isotopic compositions

The *n*-alkanes isolated from the extract of Pula sediment range from C_{16} to C_{37} with a strong predominance of C_{27}, C_{29} and C_{31} homologues (Figure 1). A similar predominance is typically observed in higher plant waxes (Kolattukudy, 1976), as illustrated in Figure 1 in the case of wheat and maize leaves. It shall be noted, however, that one of the three races known to occur in *B. braunii* (Metzger *et al.*, 1991) is characterized by an abundant production of odd carbon-numbered *n*-alkadienes with a very strong predominance of the C_{27}, C_{29} and C_{31} homologues (Fig. 1). These dienes were identified as the heptacosa-1,18 Z-diene, the nonacosa-1,20 Z-diene and the hentriaconta-1,22 Z-diene, respectively (Knights *et al.*, 1970; Metzger *et al.*, 1986). Based on their distribution, the *n*-alkanes of Pula sediment extract could be therefore derived from higher plant waxes or from the reduction of *B. braunii* *n*-alkadienes. It shall be noted, however, that the relative abundance of C_{27}, C_{29} and C_{31} *n*-alkanes from the sediment are close to the relative abundance of *B. braunii* *n*-alkadienes. Moreover, both distributions maximize at C_{27} instead of C_{29} for maize and wheat.

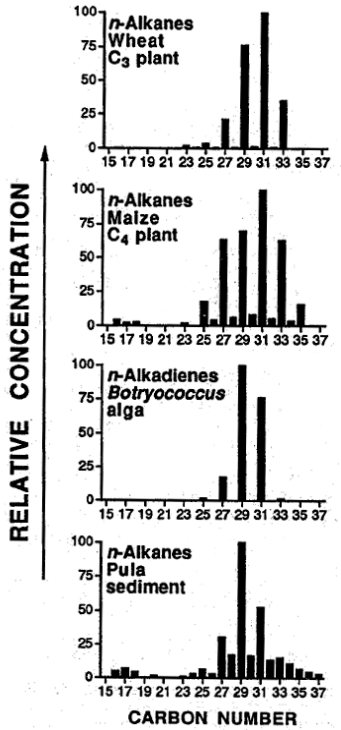

Fig. 1. Distribution of linear hydrocarbons in wheat and maize leaf waxes, extant *B. braunii* (A race) and Pula sediment extract. Relative concentrations were calculated by normalization of area obtained by gas chromatography using a flame ionization detector.

The corrected isotopic compositions observed for the C_{27}, C_{29}, C_{31} and C_{33} *n*-alkanes from maize, averaging at -18.6‰, and wheat, averaging at -35.0‰ (Table 1 and Fig. 2) are similar to those previously observed for *n*-alkanes from various C_4 and C_3 higher plants, respectively (Rieley *et al.*, 1991, 1993). Markedly different ratios, averaging at -30.5‰, are obtained for the odd-carbon numbered *n*-alkanes of Pula sediment extract. Accordingly, based on these isotope ratios, the predominant occurrence of C_{27}, C_{29} and C_{31} *n*-alkanes in Pula extract is unlikely to reflect a large input of terrestrial plant waxes. On the contrary, based both on their isotopic composition and distribution, such *n*-alkanes should indicate a major contribution of the alkadiene-producing race of *B. braunii* (A race).

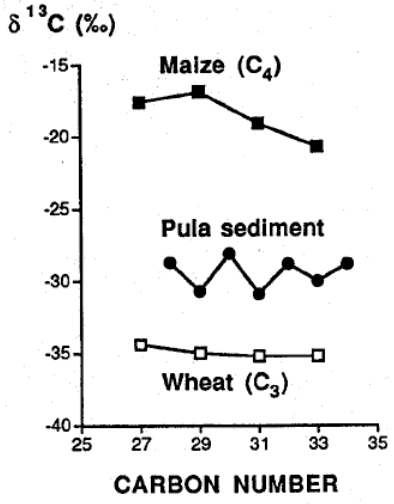

Fig. 2. Carbon isotope composition of *n*-alkanes from maize and wheat leaf waxes and Pula sediment extract. Plant values are corrected to take into account the isotopic difference between ancient and modern atmospheric CO_2 (+1.5‰).

It can be noted that *n*-alkanes in the range C_{25} to C_{35} exhibiting an odd predominance and an isotopic composition in the range -30 to -27‰ have been shown to occur in extracts of various low maturity sediments (Rieley *et al.*, 1991; Collister *et al.*, 1992; Lichtfouse and Collister, 1992; Lichtfouse *et al.*, 1994; Collister *et al.*, 1994). Based on their isotope ratios and distributions, these *n*-alkanes should not be chiefly derived from terrestrial waxes but more likely from algal lipids.

CONCLUSION

A strong predominance of odd carbon-numbered *n*-alkanes in the range C_{25} to C_{35} in sediment extracts shall not be systematically assigned to a contribution of terrestrial plant waxes. In fact, as shown here in the case of Pula oil shale *via* isotopic and molecular studies, such a feature can be also associated with a major input of microalgae. In addition, the occurrence of long chain, odd *n*-alkanes, with isotope ratios from -30‰ to -27‰, previously observed in extracts of various immature sediments is likely to reflect an important algal contribution.

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