



**Comments on "Biomarkers or not biomarkers. A new hypothesis for the origin of pristane involving derivation from methyltrimethyltridecylchromans (MTTCs) formed during diagenesis from chlorophyll and alkylphenols" from M. Li, S. R. Larter, P. Taylor, D. M. Jones, B. Bowler and M. Bjorøy**

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Recently, Li *et al.* (1995) proposed a new hypothesis for the formation of methylated methyltetramethyltridecylchromans (MTTCs; I in Fig. 1), a suite of biomarkers of as yet unknown origin. After their discovery in sediments and crude oils we (Sinninghe Damsté *et al.*, 1987) proposed that methylated MTTCs are directly biosynthesised. This conclusion was based on several observations. The limited number of structural isomers, i.e. two monomethyl, two dimethyl and one trimethyl MTTC isomers (Fig. 1), and the structural similarity (i.e. methyl substitution pattern of the most abundant isomers) with the well-known biosynthetic products, the tocopherols (II), suggested that elimination of the hydroxyl group of the tocopherols led to formation of methylated chromans. However, the identification of 6-methyl-MTTC (Ie) in a sediment, which contains a methyl substituent at the position where the hydroxyl group of tocopherols is located, and the fact that aromatic hydroxyl groups are not easily lost during early diagenesis, made a derivation from tocopherols less likely and, thus, a direct biological contribution was favoured. Li *et al.* (1995) now propose a third possible origin for methylated chromans: condensation of phenols with phytol, i.e. the way we synthesised our authentic methylated MTTC standards (Sinninghe Damsté *et al.*, 1987). At the time we did not even mention this third possible origin for a number of reasons as given below.

Li *et al.* (1995) propose that anhydrite may act as a potential dehydrating agent replacing P<sub>2</sub>O<sub>5</sub> in the condensation reaction. However, methylated chromans are found in a variety of settings and not only in evaporitic sediments (Sinninghe Damsté *et al.*, 1987, 1993). Admittedly, Li *et al.* (1995) also demonstrate that the condensation reaction proceeds without the addition of a dehydrating agent, though in much decreased yields. It should be realised, however, that methylated MTTCs are also present in

unconsolidated sediments containing substantial amounts of water (Sinninghe Damsté *et al.*, 1993 see also ten Haven *et al.*, 1990; Köster and Sinninghe Damsté, unpublished results). It is not conceivable that a chemical reaction in which the elimination of water is the driving force will take place in unconsolidated sediments.

We question whether the two reactants proposed in the condensation reaction, phytol or intact chlorophyll and phenols, will ever co-occur in sediments. Chlorophyll and phytol formed from hydrolysis of chlorophyll-a, are components found in immature sediments whereas free phenols have only been reported to occur in crude oils in low concentrations (e.g. Ioppolo *et al.*, 1992) and have not been recognised as major constituents of extracts of sediments. A reaction of phytol with free phenols thus seems implausible.

Although the co-occurrence of phytol and alkylphenols should be questioned (see above), Li *et al.* (1995) discuss in detail the isomer distribution of C<sub>3</sub> alkylated phenols in crude oils (which are certainly not known to contain phytol) and recognise that more than one C<sub>3</sub> alkylated phenol could react in the condensation reaction proposed. From a theoretical point of view 24 C<sub>3</sub> alkyl MTTC isomers (4 isopropyl, 4 propyl, 12 ethylmethyl and 3 trimethyl) can be formed from the 19 C<sub>3</sub> alkylated phenol isomers, most of them occurring in crude oils (Ioppolo *et al.*, 1992; Li *et al.*, 1995). This contrasts with the fact that only one C<sub>3</sub> alkylated MTTC (5,7,8-trimethyl, Id) has been identified so far in sediments. Similarly, in sediments deposited under normal marine conditions the methylated MTTC distribution is often dominated by this MTTC isomer since only traces of the 5,8- or 7,8-dimethyl MTTCs (Ib-c) are present in addition (Sinninghe Damsté *et al.*, 1987, 1993). This cannot be explained by the "condensation theory" since other C-1 and C-2

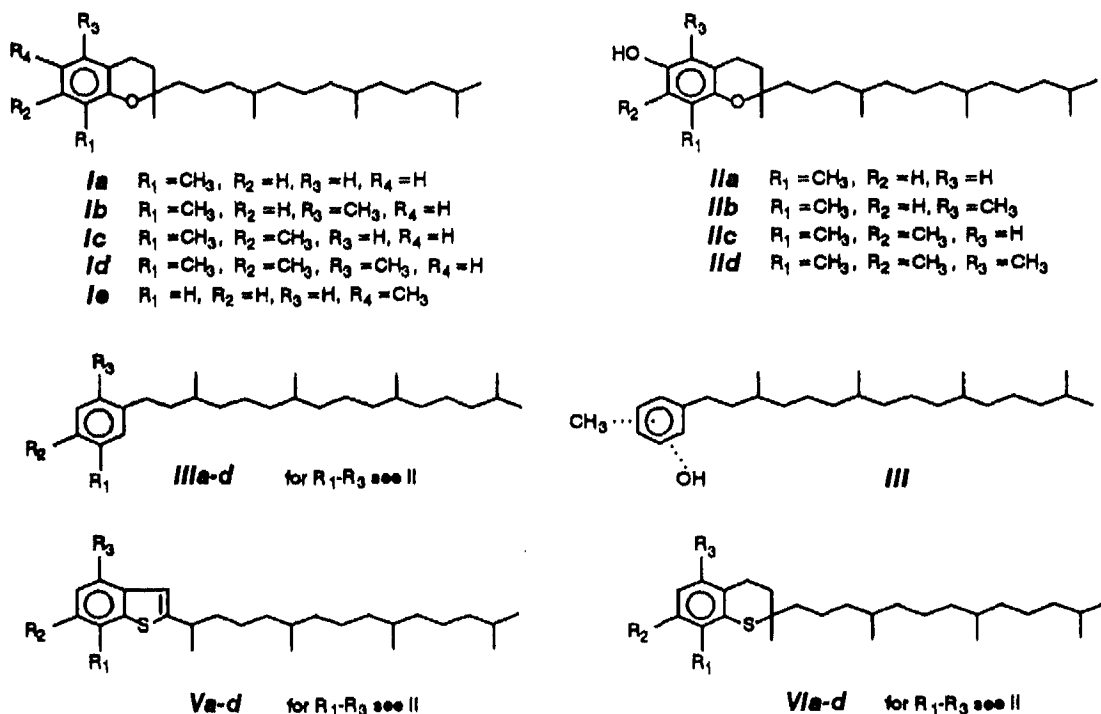


Fig. 1. Structures of compounds cited in the text. Structures III–VI have only been tentatively identified.

alkylated MTTCs would be expected to be formed as well from  $C_1$  and  $C_2$  phenols. Similarly, an unsubstituted MTTC and  $C_4$  alkylated MTTCs would be expected to be formed from condensation of phenol and  $C_4$  alkylated phenols, but have not been found to occur in nature. Li *et al.* (1995) argue that "it is possible that under diagenetic conditions with slow reaction rates, different ortho unsubstituted phenols could react selectively, resulting in the enhanced production of 5,7,8-trimethyl MTTC". However, as indicated above the enhanced production of 5,7,8-trimethyl MTTC is not adequate since almost all other methylated MTTCs, which would be possible to form, are not present.

On the basis of the formation of chlorophyll steryl esters via transesterification during grazing or very early diagenesis (King and Repeta, 1991; Prowse and Maxwell, 1991; Eckhardt *et al.*, 1992), Li *et al.* (1995) introduce the concept of "pseudo-biomarker". These components are supposed to be formed through condensation of diagenetic products of biosynthetic molecules. Li *et al.* (1995) subsequently try to provide evidence that methylated MTTCs are "pseudo-biomarkers". We object to the use of the term "pseudo-biomarker" in general and to the application of this term to methylated MTTCs in particular. The formation of steryl chlorophyll esters is not a "condensation" reaction. Hydrolysis and esterification are general, often enzyme mediated, reactions which occur ubiquitously in biological systems and in immature sediments. The formation of C-C and ether bonds, as required in the formation

of methylated MTTCs from phytol and phenols, are by no means comparable reactions and require much harsher reaction conditions. The occurrence of several groups of components, i.e. isoprenoid alkylbenzenes III (Sinninghe Damsté *et al.*, 1988), isoprenoid phenols IV (Barakat and Rullkötter, 1991), isoprenoid benzothiophenes V (Sinninghe Damsté *et al.*, 1989; Grimalt *et al.*, 1991) and isoprenoid thiochromans VI (Schaeffer, 1993), structurally related to the methylated MTTCs suggests that they are possibly derived from a common biosynthetic precursor (e.g. isoprenoid quinones as suggested previously (Sinninghe Damsté *et al.*, 1988)), which by definition makes them biomarkers.

Li *et al.* (1995) also propose that the condensation reaction may occur with macromolecularly bound phenol moieties. In this case the geological co-occurrence of the two reactants seems much more plausible since phenol moieties do occur widely in lignins and tannins. Two experiments were designed to confirm this hypothesis: flash pyrolysis of the insoluble residues of the condensation reactions of phytol with poly(*p*-vinyl)phenol and a brown coal. However, although pristenes were formed (demonstrating that some reactions took place) a large suite of other products was formed in concentrations similar to those of the pristenes. Since these other products are not encountered in pyrolysates of naturally occurring macromolecular substances, the validity of these experiments, to confirm the hypothesis that phytol can act as a source for pristane by condensation with phenols, should be questioned.

Furthermore, lignins and tannins are exclusively biosynthesised by higher plants suggesting that the condensation reactions can only operate in terrestrial environments or sediments receiving a significant contribution of terrestrial organic matter. However, the pristane precursor is abundantly present in immature kerogens from marine or hypersaline depositional settings, as revealed by the abundance of prist-1-ene in their flash pyrolysates. Also, the thermally immature Ordovician Boas Oil Shale, which predates the evolution of higher plants, contains both abundant prist-1-ene in its pyrolysate as well as the trimethyl MTTC (Koopmans and Sinninghe Damsté, in preparation), demonstrating that phenols from higher plants are neither required for the formation of methylated MTTCs nor for pristane precursors in kerogen. Finally, it should be noted that the range of activation energies required for the release of pristane from kerogen, the major problem with tocopherol as the natural precursor of pristane as recognised by the authors, will not be solved by the proposed condensation reaction of phytol with phenols. It is expected that the macromolecularly bound chroman moieties will degrade thermally in much the same way as macromolecularly bound tocopherol units (i.e. by the so-called retro Diels Alder reaction).

Although Li *et al.* try to revive the currently, as the authors state, "out of fashion" condensation theory, their attempts, at least for condensation of phytol with phenols, are not convincing to us. They end their paper with five "obvious tests of the hypothesis". Only one of them, a search for methylated MTTCs and their precursors in organisms, seems, in our view, particularly useful.

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#### REFERENCES

- Barakat A. O. and Rullkötter J. (1991) Polar lipids in sulphur-rich black shales from the Nördlinger Ries (South Germany) In: *Organic Geochemistry: Advances and Application in Energy and the Natural Environment* (Edited by D. A. C. Manning), pp. 473–475. Manchester University Press, Manchester.
- Eckhardt C. B., Pearce G. E. S., Keely B. J., Kowalewska G., Jaffe R. and Maxwell J. R. (1992) A widespread chlorophyll transformation pathway in the aquatic environment. *Org. Geochem.* **19**, 217–227.
- Grimalt J. O., Grifoll M., Solanas A. M. and Albaiges J. (1991) Microbial degradation of marine evaporitic crude oils. *Geochim. Cosmochim. Acta* **55**, 1903–1913.
- Ioppolo M., Alexander R. and Kagi R.I. (1992) Identification and analysis of C<sub>6</sub>–C<sub>7</sub> phenols in some Australian crude oils. *Org. Geochem.* **18**, 603–609.
- King L. and Repeta D. J. (1991) Novel pyropheophorbide steryl esters in Black Sea sediments. *Geochim. Cosmochim. Acta* **55**, 2067–2074.
- Li M., Larter S. R., Taylor P., Jones D. M., Bowler B. and Bjørøy M. (1995) Biomarkers or not biomarkers? A new hypothesis for the origin of pristane involving derivation from methyltrimethyltridecylchromans (MTTCs) formed during diagenesis from chlorophyll and alkylphenols. *Org. Geochem.* **23**, 159–167.
- Prowse W. A. G. and Maxwell J. R. (1991) High molecular weight chlorins in a lacustrine shale. *Org. Geochem.* **17**, 877–886.
- Schaeffer P. (1993) *Marquers Biologiques de Milieux Evaporitiques*. Ph.D. Thesis, University of Strasbourg, 339pp.
- Sinninghe Damsté J. S., de Leeuw J. W., Kock-van Dalen A. C., de Zeeuw M. A., de Large F., Rijpstra W. I. C. and Schenck P. A. (1987) The occurrence and identification of series of organic sulphur compounds in oils and sediment extracts. I. A study of Roze! Point Oil (USA). *Geochim. Cosmochim. Acta* **51**, 2369–2391.
- Sinninghe Damsté J. S., Kock-van Dalen A. C. and de Leeuw J. W. (1988) Identification of isoprenoid alkylbenzenes in sediments and crude oils. *Geochim. Cosmochim. Acta* **52**, 2671–2677.
- Sinninghe Damsté J. S., Rijpstra W. I. C., de Leeuw J. W. and Schenck P. A. (1989) The occurrence and identification of series of organic sulphur compounds in oils and sediment extracts. II. Their presence in samples from hypersaline and non-hypersaline depositional environments and their possible application as source, palaeoenvironmental and maturity indicators. *Geochim. Cosmochim. Acta* **53**, 1423–1441.
- Sinninghe Damsté J. S., Keely B., Betts S., Baas M., Maxwell J. R. and de Leeuw J. W. (1993) Variations in abundances and distributions of isoprenoid chromans and long-chain alkylbenzenes in sediments of the Mulhouse Basin: A molecular sedimentary record of palaeosalinity. *Org. Geochem.* **20**, 1201–1215.
- ten Haven H. L., Rullkötter J., Sinninghe Damsté J. S. and de Leeuw J. W. (1990) Distribution of organic sulphur compounds in Mesozoic and Cenozoic deep-sea sediments from the Atlantic and Pacific Oceans and the Gulf of California. In *Geochemistry of Sulfur in Fossil Fuels* (Edited by W. L. Orr and C. M. White), pp. 613–632. ACS Symp. Ser. 429, Am. Chem. Soc., Washington.