

HOMOHOPEANE IN MESSEL OIL SHALE :
FIRST IDENTIFICATION OF A C₃₁ PENTACYCLIC TRITERPANE IN NATURE
Bacterial origin of some triterpanes in ancient sediments?

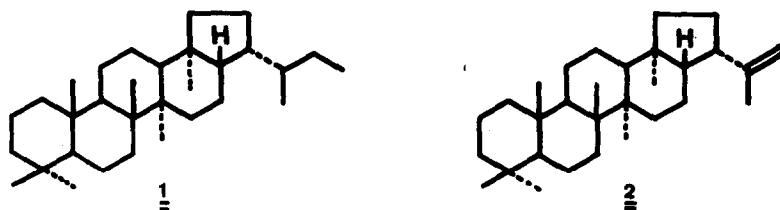
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(Received in UK 3 July 1972; accepted for publication 8 August 1972)

The Messel oil shale, located near Darmstadt, Germany, is a terrestrial organic-rich sediment of Eocene age ($\sim 50 \times 10^6$ years), which formed in shallow lakes and has been subjected to very mild geothermal conditions¹. Examination of the organic solvent-soluble compounds of the shale has led to the identification of several biological markers, including triterpenoid and steroid alcohols and ketones, as well as the regular isoprenoid alkanes with 15, 16, 18, 19 and 20 carbon atoms².

In a recent study of the branched and cyclic alkane fraction, we reported the identification of the tetraterpene lycopane, probably a reduction product of lycopene under geological conditions, and showed the presence of a series of pentacyclic triterpanes (from C₂₇ to C₃₂), presumably of the hopane type, and including hopane itself³.

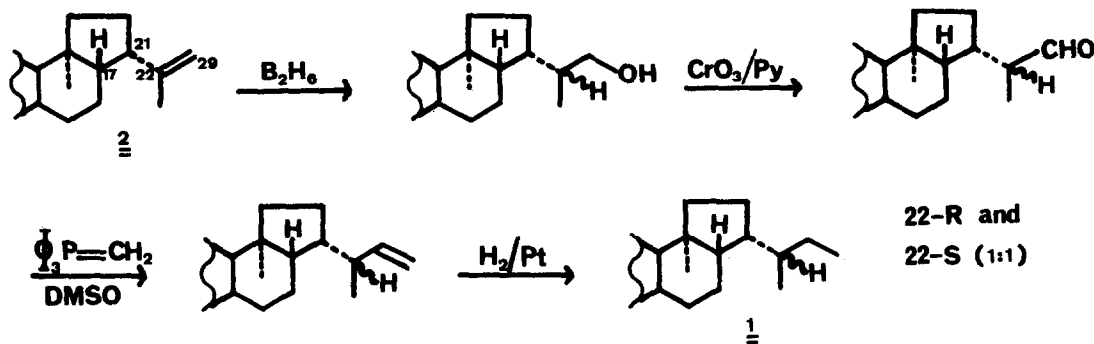
In the present study, a C₃₁ triterpane, a homohopane (1) has been identified as one of the major components of the branched and cyclic alkane fraction. The identification is based on capillary column g.l.c. retention data and coinjection and gc-ms comparison with an authentic standard prepared from diploptene (hop-22(29)-ene) (2).



The benzene-methanol (3/1) extract of the powdered rock was chromatographed over silica gel and silica gel impregnated with 10% AgNO₃ to yield 170 ppm of total alkanes. These alkanes were treated with 5 Å molecular sieve to separate the n-alkanes from the branched and cyclic alkanes. The latter (54 ppm) were then analysed by packed column g.l.c. and g.c.m.s. (2m x 3 mm o.d. 2% Dexsil), preparative g.l.c. (3m x 6 mm o.d. 5% SE 30), and m.s., capillary column g.l.c. (23m x 0.25 mm i.d. OV 101; 45m x 0.25 mm i.d. Apiezon L). The mass spectrum of one of the main components (7-8% of the branched and cyclic alkanes, i.e. 3-4 ppm) was identical

with that of the homohopane (1) standard. The presence of hopane itself was confirmed in the same way.

The homohopane was synthesised in good yield as a 1/1 mixture of diastereomers starting from diploptene (hop-22(29)-ene) by the following scheme



The resulting two diastereomers could be separated on either capillary column and only one of these peaks reinforced that assigned to homohopane in the Messel shale.

The presence in sedimentary rocks and petroleum of triterpanes with more than 30 carbon atoms in their skeleton has been noted by several authors⁴. For example, triterpanes with 31 and 32 carbon atoms are present in trace amounts in the Eocene Green River oil shale from Colorado and Wyoming, U.S.A.; but so far none of these compounds has been fully characterized. The unambiguous identification of homohopane as one of the main components of the Messel alkane fraction poses an interesting problem, because pentacyclic triterpanes with more than 30 carbon atoms have so far never been isolated from biological material. Several explanations may be considered :

1) the compound arises from biologically derived 3-oxygenated triterpenoids by reduction of the function and subsequent methylation steps. This hypothesis, although it cannot be completely excluded, seems improbable for two reasons : firstly, 3-oxygenated triterpenes of the hopane series are not present in substantial amounts in the shale, and secondly, no trace of arborane is present in the alkane fraction of the shale. Since the corresponding 3β -alcohol isocarborinol is one of the main components of the shale², this shows that reduction of oxygenated functions has not been a very efficient diagenetic maturation process in the Messel shale.

2) homohopane was formed by a non-biological transalkylation process involving radical coupling of a molecule such as diploptene (2) to longer chains or to the kerogen matrix, followed by C-C bond cleavage under geological conditions⁵. Although such a mechanism could be responsible for the formation of the complex mixtures of alkyl porphyrins which have been detected in certain sediments⁶, it would almost certainly require elevated temperatures and would yield a mixture of both 22-diastereomers of homohopane. However, only one diastereomer is present in the shale, and this process now seems improbable, in this case.

3) the compound originally entered the sediment as a 3-desoxy C_{30} triterpene and was subsequently microbiologically methylated. Such desoxy-compounds have been detected in ferns⁷, and diploptene itself has been shown recently to be a widespread constituent of

prokaryotic organisms such as blue green algae and bacteria⁸. Microbiological methylation reactions, unreported so far for pentacyclic triterpenes, are known to occur at the C-24 in the side chain during the biosynthesis of sterols. This hypothesis is strongly supported by two observations : firstly, prokaryotes contain only small amounts of sterols, but do contain 4 α -methylsterols⁹ and from previous work^{2c} the Messel oil shale contains very little sterols but does contain substantial amounts of 4 α -methyl stanols and stanones. Secondly, the shale almost certainly contains hop-17(21)-ene³ which is itself a bacterial constituent^{8b} and can be formed easily by isomerization of diploptene under mild conditions.

The data thus point towards a microbiological origin for the homohopane isolated from the Messel oil shale. This result is important, because so far most of the pentacyclic triterpanes found in sediments and petroleum have been considered, previously at least, as paleoecological markers indicating a higher plant origin for part of the organic matter present in these sources. A possible exception seems to be the presence in the Green River shale of the triterpane gammacerane (tetrahymane)¹⁰, probably a reduction product of the corresponding alcohol tetrahymanol, a constituent of ciliated protozoa, which is itself present in trace amounts in the Green River shale¹¹. In the Messel shale all the principal triterpanes appear to be of the hopane type; this is also true for a shale from Bouxwiller (France) which is geologically related to Messel⁵. Hopane itself appears to be one of the main triterpanes in the Green River shale^{4a} and triterpanes of the hopane series are widely present in petroleum¹². Our results, combined with the most recent results on microbial chemistry, suggest that a significant proportion of the triterpanes found in sediments and oil could be of bacterial origin, as inferred by Bird *et al.*^{8a}.

Further work on identification of the other components of the triterpane series, as well as on elucidation of the methylation mechanism is in progress.

The authors wish to thank the YTONG AG and the management of the Messel mine for shale samples; Professor H. AGETA for a sample of diploptene; M. P. ARPINO for help in the capillary column work and M. G. TELLER for mass spectra.

Part of this work has been supported by ELF-ERAP and PRF-ACS (3286-A2). The Strasbourg group is part of a "Laboratoire Associé au CNRS".

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