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Heating of Hop-17(21)-ene in Molten Sulphur: a Route to New Sedimentary Biomarkers of the Hopane Series?

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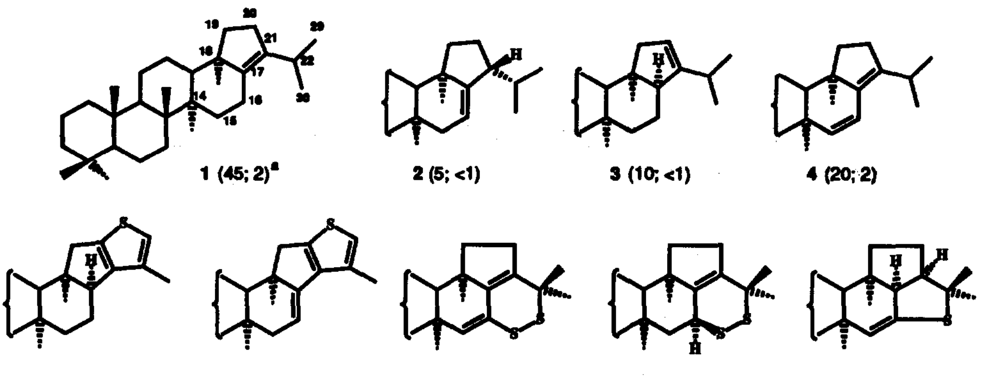
Abstract: On heating in liquid sulphur, the unsaturated hopanoid hop-17(21)-ene **1** was converted to a major extent into the series of organo-sulphur products **5-9**, all of interest as potential sedimentary biomarkers.

Since their first recognition almost ten years ago,[1a] sulphur-containing geohopanoids have been occasionally reported from immature sediments of various origins, so that they are now emerging as a noteworthy and probably much overlooked family of organo-sulphur biomarkers.[1] Those include not only C_{35} geohopanoids with thiophene shaped side-chains, which most probably result from the direct incorporation of sulphur (or a related inorganic species) into the polyhydroxylated side-chain of bacteriohopanoid precursors, but also geohopanoids possessing an atom of sulphur anchored to their triterpenic framework, probably representing the chemical fossils of biohopanoids with unsaturated or hydroxylated skeletons. In order to throw light on the formation of these latter compounds and to have access to reference samples of geochemical value, we decided to extend our previous heating experiments of saturated biohopanoids in molten sulphur[2] to the more reactive hop-17(21)-ene **1**, a hopanoid found in some ferns[3] which in addition represents a thermodynamically more stable isomer of the widespread hop-22(29)-ene (or diploptene).
The greater reactivity of **1** was confirmed in a preliminary experiment: when this unsaturated triterpene (5mg) was heated in an excess of molten sulphur (25mg) under the conditions selected to study the isomerization at C-17 of the corresponding saturated 17β,21β-hopane,[2] *i.e.* 3h at 240°C, only traces (<5%) of a complex mixture of apolar products soluble in $CHCl_3$ were formed next to a major black residue (5mg) of probably polymeric nature.

Switching thus to milder conditions enabled us to identify, next to the starting hopene **1** and two of its isomers **2** and **3**, the diene **4** and the series of organo-sulphur hopanoids **5-9**.



1 (45; 2)[a] 2 (5; <1) 3 (10; <1) 4 (20; 2)

5 (6; 30) 6 (2; 20) 7 (<1; 8) 8 (6; 12) 9 (1; 22)

Hopanoids resulting from heating experiments of hop-17(21)-ene 1 in liquid sulphur.

[a] The first figure in parentheses refers to the percentage of product obtained under milder conditions (*i.e.*: 30min at 200°C), the second to 20min of heating at 220°C.

Typical experiment. Hop-17(21)-ene 1 (10mg, >99.5% in gc) was heated in a closed vial under argon gas in an excess of sulphur (45mg) using one of the above conditions. After cooling, the brownish medium was dissolved into CHCl$_3$ and submitted to a short column of active copper powder in order to get rid of any remaining sulphur. A first SiO$_2$ tlc using hexane as eluent led to three main fractions at R$_f$=0.60, 0.35 and 0.20. The first two could be directly further purified by tlc over SiO$_2$ impregnated with 10% AgNO$_3$ into respectively 1 (R$_f$=0.30), 2 (R$_f$=0.65), 3 (R$_f$=0.45) and 4 (R$_f$=0.45) using hexane:toluene (96:4, v:v) as eluent and 5 (R$_f$=0.15) and 6 (R$_f$=0.10) with hexane:toluene (9:1, v:v), whereas the third fraction required a further tlc separation over SiO$_2$ using hexane and three migrations, into two subfractions of R$_f$=0.40 and 0.45 prior to final AgNO$_3$ SiO$_2$ tlc using normal distilled CHCl$_3$ as eluent and two migrations to get 7-9 pure as determined by gc (>98%).

Thiophene hopanoids 5 and 6 proved identical (gc, ^{1}H-nmr, ms) with the two organosulphur products resulting from the action of sulphur on the saturated 17β,21β-hopane.[2] With larger amounts to our disposal, ^{13}C-nmr spectra of 5 and 6 were recorded, showing in particular in accordance with their structure respectively eight and nine quaternary carbon atoms.[4] Furthermore, in the case of 5, the 17α configuration (previously supported by correlation with 17α,21β-hopane after Raney nickel treatment and catalytic hydrogenation)[2] was ascertained in ^{1}H-nmr by nOe enhancement between the protons of the 18α-methyl group and H-17. Identification of 2 and 4 was also straightforward as analytical data (^{1}H-nmr and mp for both, ms for 2 and uv for 4) have been already published.[5,6] The poorly debated 21β-configuration in hop-16-ene 2[4] was in addition confirmed by its conversion into the saturated 17α,21β-hopane after mild catalytic hydrogenation.[7] Identification of 17α-hop-20-ene 3 was supported in ^{1}H-nmr[4] by a characteristic narrow multiplet at 5.21ppm corresponding to 20-H and in ms[7] by the ratio of the peak intensities at m/z=191 and 189, both resulting from the characteristic ring C-cleavage of the molecule,[8] similar to the one previously reported for 17α-hop-22(29)-ene and typical of related hopanoids of the 17α series.[9] Confirmation of the latter configuration resulted, as in the case of 2, from its hydrogenation product.[7] As independent support for structure 4 as well as to the characteristic nmr signal of 20-H in hopene 3, 17β,21β-epoxyhopane was converted through 1N HCl treatment in $CHCl_3$:MeOH (1:2, v:v) to give as major products (70%) not only into the expected transoid diene 4,[6] but also into the isomeric hopa-16,20-diene which exhibited the same typical nmr signal for 20-H at 5.46ppm.

Sulphur-containing hopanoids 7 and 9 were easily identified on the basis of their explicit ^{1}H-nmr spectra as well as their informative gc-ms analyses.[4] Indeed in ms both compounds showed apart from the molecular ion, fragmentations of diagnostic value like the ones originating from ring-C cleavage[8] at m/z=249 and 220 for respectively 7 and 9, and, in the case of 7, the base peak at m/z=423 due to the loss of an atom of sulphur and a methyl group, corresponding probably to a thiophenic fragment. Structure 7 was further confirmed in uv spectroscopy as it presented an absorption similar to that of its non-sulphurated homologue 4 (λ_{max}=247 and 253nm). 17α,21α Configuration of sulfide 9 was supported not only by its conversion into 17α,21α-hopane (gc, ^{1}H-nmr) after Raney nickel desulphuration and PtO_2 catalytic hydrogenation but also in nmr by nOe experiments. Indeed, irradiation of H-21 at 2.24ppm gave a strong enhancement (*ca.* 15%) of H-17, indicating the *cis* junction between the two five-membered rings, whereas irradiation of the latter hydrogen resulted in a significant nOe effect not only, as expected, on H-21, but also on the neighbouring 18α-CH_3. Identification of disulfide 8 was hampered by its instability in gc-ms, probably losing S_2 *via* a *retro*-Diels-Alder reaction,[10] resulting in a broad peak in gc and showing in ms at higher masses only one ion at m/z=408 corresponding to a hopadiene. Direct inlet ms under chemical ionization (Ci-ms) using isobutane as reacting gas enabled nevertheless the recognition of the true molecular weight from the two M+1 and M+57 peaks of diagnostic value.[4] Analysis of 8 was completed in ^{1}H-nmr with in particular, as in the case of 7 and 9, two deshielded singlets accounting for the methyl groups adjacent to the sulphur atom and interestingly a remarkable homoallylic coupling constant of 3.5Hz between one of its 20-H and its 16-H;[4] irradiation of the latter hydrogen resulted in a strong nOe effect on the neighbouring 14α-CH_3, establishing thus its 16α configuration.

Without entering into the discussion of the ionic or radical nature of these transformations, contrary to our first study on the saturated hopane, action of sulphur on hop-17(21)-ene follows here a more classical chemistry, involving as expected isomerization and dehydrogenation reactions as well as sulphur incorporations. Although to our knowledge none of the products described here has been yet detected in sediments, hydrogenated derivatives of 5 and 6 have already been isolated.[1b] In the case in particular of the other sulphur-containing hopanoids 7-9, we predict their existence as such or as closely related substances and hope they would serve as useful references to geochemists. These experiments will be repeated in the presence of an acidic catalyst, like a sedimentary clay, as well as on other unsaturated hopanoids in order to try to obtain milder and thus more geomimetic conditions and also to obtain access for instance to demethylated aromatic geohopanoids, which could not be detected in the present work.

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References and Notes

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4. *Selected analytical data:*

3; ^{1}H-nmr (CDCl$_3$, 250 MHz): δ, 0.791 (3H, s), 0.811 (3H, s), 0.844 (3H, s), 0.885 (3H, s), 0.932 (3H, d, J=6.5Hz), 1.014 (3H, s), 1.032 (3H, s), 1.090 (3H, d, J=6.5Hz), 1.9 (1H, m, allylic proton), 2.2 (3H, m, allylic protons), 5.2 (1H, narrow m, 20-H); ms, m/z: 410 (M^{+}, 23%), 395 (6%), 367 (6%), 204 (25%),191 (100%), 189 (27%).

5; ^{13}C-nmr (CDCl$_3$, 63MHz): δ, 14.7, 16.1, 16.2, 18.3, 18.6, 18.7, 21.6 (2 carbons), 23.0, 23.9, 24.5, 29.2, 33.1, 33.3^{a}, 33.4, 37.5^{a}, 40.3, 40.8, 41.1^{a}, 42.1, 42.2^{a}, 46.0, 49.2, 51.2, 52.2^{a}, 56.5, 122.1, 132.9^{a}, 140.7^{a}, 148.0^{a}.

6; ^{13}C-nmr (CDCl$_3$, 63MHz): δ, 14.8, 15.8, 17.1, 18.5, 18.7, 19.0, 20.7, 21.7, 23.0, 23.6, 33.2, 33.3^{a}, 33.5, 34.1, 37.4^{a}, 40.3, 40.7^{a}, 42.0^{a}, 42.1, 43.0, 46.1, 50.2, 52.3^{a}, 56.2, 113.8, 123.2, 131.5^{a}, 144.0^{a}, 144.3^{a}, 145.3^{a}.

7; ^{1}H-nmr (CDCl$_3$, 400MHz): δ, 0.789 (3H, s), 0.799 (3H, s), 0.856 (3H, s), 0.876 (3H,s) 0.885 (3H, s), 1.170 (3H,s, 14α-CH$_3$), 1.466 (3H, s, 22-CH$_3$), 1.544 (3H, s, 22-CH$_3$), 2.32 (1H, dd, J=17 & 9Hz, 20-Ha), 2.54 (1H, ddd, J=17 & 10 & 7Hz, 20-Hb), 5.81 (1H, s, H-15); ms, m/z: 470 (M^{+}, 12%), 455 (35%), 438 (22%), 423 (100%), 249 (92%), 231 514%), 217 (39%).

8; ^{1}H-nmr (CDCl$_3$, 250MHz): δ, 0.794 (3H,s), 0.836 (3H, s), 0.855 (3H, s), 0.903 (3H, s), 0.964 (3H, s), 1.097 (3H, s, 14α-CH$_3$), 1.393 (3H, s, 22-CH$_3$), 1.606 (3H, s, 22-CH$_3$), 2.22 (1H, dd, J=16 & 9Hz, 20-Ha), 2.43 (1H, dddd, J=16 & 10.5 & 7.5 & 3.5Hz, 20-Hb), 3.77 (1H, m, 16α-H); ^{13}C-nmr (CDCl$_3$, 63MHz): δ, 15.6, 16.3, 16.4, 18.6, 18.7, 19.7, 21.2, 21.6, 24.3, 26.0, 27.7, 30.6, 33.3^{a}, 33.4, 33.5, 37.4, 37.5^{a}, 38.9, 40.5, 40.9, 42.1, 42.2^{a}, 42.8^{a}, 43.5^{a}, 48.9, 50.9, 51.8^{a}, 56.3, 137.6^{a}, 141.4^{a}; ci-ms (*i*-butane), m/z: 529 (M^{+}+57, 13%), 473 (M^{+}+1, 100%), 408 (38%).

9; ^{1}H-nmr (CDCl$_3$, 250MHz): δ, 0.801 (3H, s), 0.811 (3H, s), 0.855 (3H, s), 0.921 (3H, s), 0.971 (3H, s, 18α-CH$_3$), 1.076 (3H, s, 14α-CH$_3$), 1.381 (3H, s, 22-CH$_3$), 1.530 (3H, s, 22-CH$_3$), 2.24 (1H, dt, J=10 & 6.5Hz, H-21), 2.71 (1H, dd, J=7 & 3Hz, H-17), 5.66 (1H, d, J=3Hz, H-15); ^{13}C-nmr (CDCl$_3$, 63MHz): δ, 14.3, 16.1, 17.3, 18.5, 18.8, 21.4, 21.7, 24.8, 27.0, 27.5, 28.8, 33.3^{a}, 33.4, 33.5, 35.0, 37.6^{a}, 40.3, 40.9^{a}, 42.1, 44.5^{a}, 45.6, 46.3^{a}, 47.9, 50.6, 53.8^{a}, 56.5, 58.8, 58.9, 125.5, 139.3^{a}; ms, m/z: 440 (M^{+}, 64%), 425 (8%), 233 (48%), 220 (100%), 191 (12%).

[a] In ^{13}C-nmr, specifies quaternary carbon atoms.

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