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2α-Methylhopanoids: First Recognition in the Bacterium *Methylobacterium organophilum* and Obtention via Sulphur Induced Isomerization of 2β-Methylhopanoids.

An account for their presence in sediments.

P Stampf, D Herrmann, P Bisseret and M Rohmer*

Ecole Nationale Supérieure de Chimie de Mulhouse, 3 rue A Werner, 68093 Mulhouse Cedex, France

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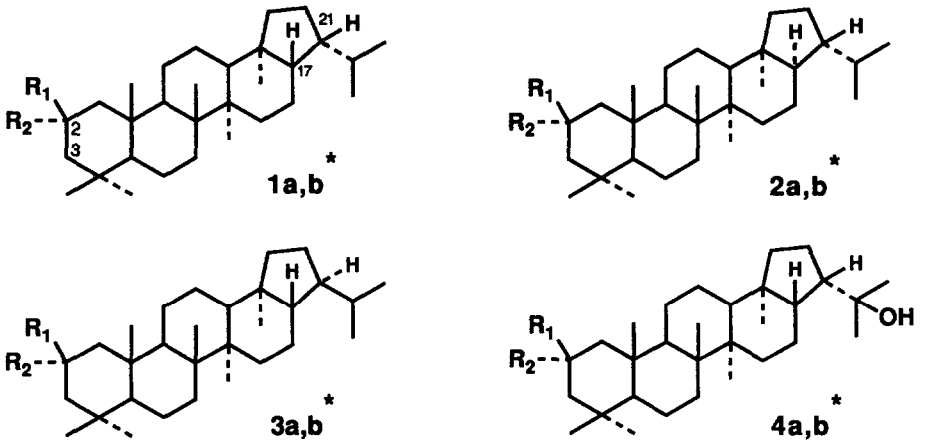
Abstract First recognition of 2α-methyldiplopterol from the bacterium *Methylobacterium organophilum* and sulphur induced isomerization of 2β-methylhopanoids into their 2α-methyl isomers suggest a dual origin for the sedimentary 2α-methylhopanoids

Triterpenoids from the hopane family isolated from living organisms ("biohopanoids") are typically derived from the C_{30} 17β,21β framework **1** (Scheme 1) [1] They are the precursors of the many hopanoids encountered in sediments ("geohopanoids") which often possess the thermodynamically more stable skeleton **2**, and to a lesser extent **3** of respective 17α,21β and 17β,21α configurations [2] Beside these major compounds, minor members possessing an additional methyl group attached at position 2β or 3β of their 17β,21β skeleton have been isolated from a few bacteria scattered through several taxonomic groups [1a,3] These are thus the putative precursors of the widespread geohopanoids characterized by an additional methyl group at position 2 or 3 of their 17α,21β skeleton. Only recently have the configurations at C-2 and C-3 been discussed. In the case of 3-methylhopanoids, compounds bearing the thermodynamically favoured equatorial 3β-methyl group were only encountered, whereas a mixture of both 2α- and 2β-methylhopanoids appeared in younger sediments [4,5] In more mature geological samples, only the 2α-methyl isomers were found,[4,5] illustrating thus the transformation into the most stable series during the diagenesis as early recognized for the apparent 17β/17α epimerization [2] To account for this latter isomerization, we pointed out recently the good epimerization power of sulphur, an element often present in sediments, at high temperatures [6] We present here further heating simulations in liquid sulphur to investigate the 2β-methyl/2α-methyl isomerization using mainly 2β-methyl-17α-hopane **2a**, with the

thermodynamically most stable configuration at C-17, as a model compound and the first identification of 2α-methyldiplopterol from the bacterium *Methylobacterium organophilum*.



Scheme 1. *Non indexed figures refer to $R_1=R_2=H$, **a** indexed ones to $R_1=CH_3$, $R_2=H$ and **b** indexed ones to $R_1=H$, $R_2=CH_3$

2α-METHYLDIPLOPTEROL IN *Methylobacterium organophilum* IDENTIFICATION AND BIOSYNTHESIS

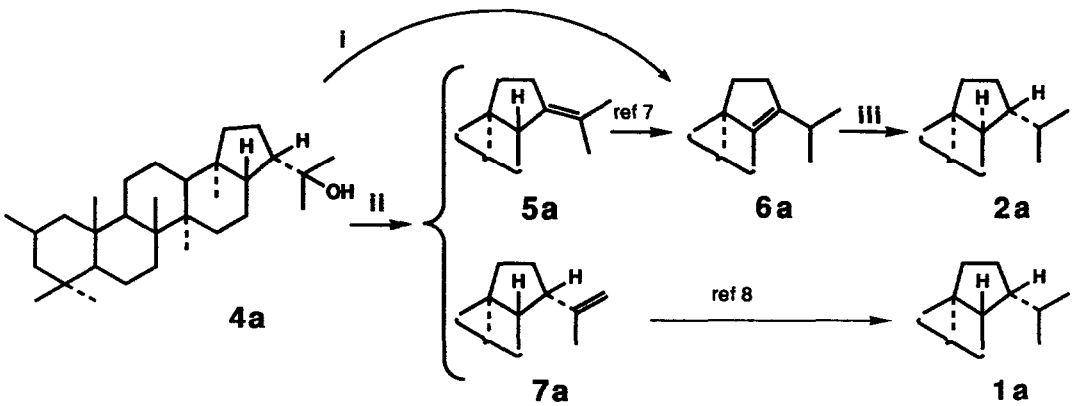
With larger amounts (20mg) of tlc-purified 2β-methyldiplopterol **4a**, isolated from *M organophilum* and reported as pure when the bacterium was grown on the Hestrin and Schramm medium,[3a] we repeated the ^{13}C-nmr analysis and detected *ca* 5% of its 2α-methyl isomer **4b** by comparison with the spectrum now available of synthetic 2α-methyldiplopterol Further improvement of the gc analyses permitted also to detect this minor compound possessing a slightly longer retention time than that of 2β-methyldiplopterol

Methylation of hydrocarbon skeletons occurs usually via an olefinic precursor, *S*-adenosylmethionine being the methyl donor, as shown for instance for the methylation reactions of phytosterol side-chains. Feeding experiments with ($^2H_3$)-methylmethionine showed that the labelled methyl group was transferred with its three deuterium atoms into 2β-methyldiplopterol.[3c] Although an unsaturated hopanoid could not be detected until now, this confirmed the role of methionine as precursor of the methyl donor and excluded intermediates with methylene or cyclopropyl groups in this biosynthetic pathway Reinvestigation by gc-ms of this deuterated 2β-methyldiplopterol sample using the new gc procedure permitted again to identify 2α-methyldiplopterol Next to non-labelled 2α-methyldiplopterol synthesized from non-labelled carbon source, only the trideuteriated analogue arising from labelled methionine, and no mono- and dideuteriated compounds, could be detected The conclusions concer-

ning the structures of possible intermediates for the biosynthesis of 2α-methylhopanoids are thus similar to those described for the formation of their 2β-methyl isomers It has still to be determined whether there are two similar, but separate and stereoselective biosynthetic pathways, or one single enzymatic reaction sequence with low stereoselectivity.

SYNTHESIS OF 2β-METHYL AND 2α-METHYLHOPANOIDS

2β-Methyl-17β- and 17α-hopanes **1a** and **2a**, both accompanied by 5% of their 2α-methyl isomer, *i e* reflecting the natural isomeric ratio at C-2 in *M organophilum*, were obtained from natural 2β-methyldiplopterol **4a** according to classical methods (Scheme 2)



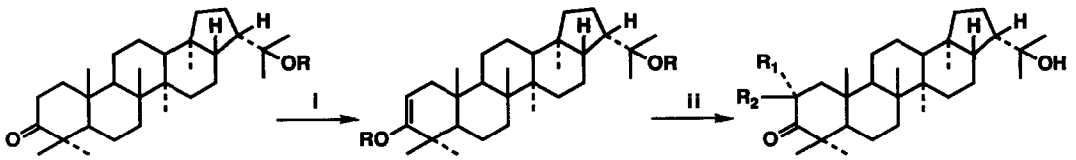
Scheme 2 Synthesis of 2β-methyl-17β- and 17α-hopanes **1a** and **2a** starting from 2β-methyldiplopterol **4a** isolated from *M organophilum*, each compound is accompanied by traces (5%) of 2α-methyl isomer i, $HClO_4$, HCO_2H, $CHCl_3$, 20°C, 10min, ii, $SOCl_2$, Py, 15 min, 0°C, iii, H_2, $Pd(OH)_2$, AcOH, AcOEt, $HClO_4$, 18h, 20°C

After thionyl chloride dehydration of 2β-methyldiplopterol **4a** in the presence of pyridine,[9] silver nitrate tlc purification yielded the two olefins **5a** and **7a** in a 2 3 proportion, giving thus comparable results as the Ac_2O/K_2CO_3 mediated dehydration reported on diplopterol **4** [10] 2β-Methyldiploptene **7a** on the one hand was directly hydrogenated using Adam's catalyst into the required 2β-methyl-17β-hopane **1a**.[8] 2β-Methylhop-21-ene **5a** on the other hand was converted to the $\Delta^{17(21)}$-unsaturated compound **6a** under acidic conditions,[7] which then was hydrogenated to the 17α isomer **2a** using either Adam's or Pearlman's catalyst, both under strong acidic conditions [11] Use of a palladium catalyst proved fruitful, as recognized by Summons and Jahnke with Pd/C,[4a] yielding a better stereospecific conversion into **2a**. When required, **4a** was efficiently directly dehydrated into **6a** in the presence of perchloric acid

2α-Methyl-17β- and 17α-hopanes **1b** and **2b** were prepared from 22-hydroxyhopan-3-one **8a** according to the improved methylation procedure shown in Scheme 3 The initial method on small quantities of **8a** (<0.1mmole)[3a] yielding rather irrepro-

ducible results and in particular often substantial amount of 2,2'-dimethylated compound.

Monomethylation of the silylated enol ether **9**[13] occurred with a satisfactory reproducible α-stereoselectivity (80%) and led after restoration of the hydroxy group and reverse phase hplc purification to 2α-methyl-22-hydroxyhopan-3-one **10b** which after Wolff-Kishner reduction afforded pure 2α-methyldiplopterol **4b**. Handling of this tertiary alcohol as described for the 2β-methyl epimer (Scheme 2) gave the required hydrocarbons **1b** and **2b**.



8a, R=H, **8b**, R=TMS — **9**, R= TMS — **10a**, R_1=H, R_2=CH_3; **10b**, R_1=CH_3, R_2=H

Scheme 3. Synthesis of 2α-methyl-17β- and 17α-hopanes **1b** and **2b** from 22-hydroxyhopan-3-one **8a**. i, DMF, NEt_3, $SiMe_3Cl$. ii, *n* $Bu_4N^+F^-$, MeI, THF then MeOH, HCl. iii, Wolff-Kishner[12] then hplc, iv, $SOCl_2$, Py, 15min, O°C Compounds **1b** and **2b** were obtained from **10b** analogous to scheme 2

ISOMERIZATION IN LIQUID SULPHUR

2β-Methyl-17β- and 17α-hopanes **1a** and **2a**, both accompanied by a trace amounts (5%) of their 2α-methyl epimers, thus reflecting the natural epimeric mixture, were heated with a large excess of molten sulphur under argon gas in closed pyrex-glass vials (Table 1)

To put light on the isomerization at C-2, experiments were first carried out on **2a** with the 17α configuration known to be stable during heating with liquid sulphur At the lowest temperature tested (Table 1, entry 1), the apolar hopanoid mixture was found to be almost identical in composition to the starting product with in particular no enhancement in 2α-methyl isomer **2b**, only the odor of H_2S and the appearance of a brown polar material indicated action of sulphur. At 240°C, the same composition was obtained both after 3h and 24h of heating, revealing a modest enhancement of **2b** and the limits of the epimerization power of sulphur. Heated under typical conditions (3h at 240°C), 2β-methyl-17β-hopane **1a** yielded mostly a hopanoid mixture of predominant 17α,21β and to a lesser extent 17β,21α configuration, both with a major (85%) 2β-methyl configuration (Table 1, entry 4).

Table 1: Heating of hopanoids **1a** and **2a** in liquid sulphur [a]

Entry	Compound	Hopanoid /Sulphur (mg)	T(°C)	t(h)	Yield [b] %	Composition[c] %
1	**2a**	3 / 30	200	3 h	65	**2a** (90%), **2b** (5%), unknowns (5%)
2	**2a**	3 / 30	240	3 h	50	**2a** (80%), **2b** (15%), unknowns (5%)
3	**2a**	3 / 30	240	24h	35	**2a** (80%), **2b** (15%), unknowns (5%)
4	**1a**	6 / 30	240	3 h	45	**1a,b** (6%)[d], **2a,b** (75%)[d], **3a,b** (17%)[d], minor compounds (2%)[e]

a, for experimental conditions, see ref 6, b, for apolar compounds, the rest represents a more polar brown material, c, analyses performed thanks to synthetic reference materials, % estimated both by gc and ^{13}C-nmr for entries 1-3 and by gc only for entry 4, d, 2α-methyl/2β-methyl isomeric ratio = *ca* 15 85 (gc), e, see ref 6

DISCUSSION

The detection of 2α-methyldiplopterol in *M organophilum* represents the first record in a living organism of a series so far reported only in some sediments 2α-Methylhopanoids have only been so far reported from hypersaline and carbonate sediments[4] and from bitumen samples from archeological sites [5] This apparent lack of methylation stereospecificity has to be compared to the usual occurrence of both (24*R*)- and (24*S*)-methylsterols in many plant species. Indeed, the biosynthesis of 2β-methylhopanoids was shown to involve *L*-methionine,[3c] most probably *via S*-adenosylmethionine as a methyl donor, like in higher plants As it is reasonable to anticipate the presence of 2α-methylhopanoids in other prokaryotes, this finding in itself could account for the occurrence of this series in sediments but not for its predominance compared to 2β-methylhopanoids, unless the formers appear much more widespread in bacteria. To investigate the possibility of diagenetic-induced epimerization of 2β-methyl into the 2α-methyl group, we extended our experiments of sulphur-induced isomerization at C-17 to carbon C-2 of methylhopanoids The epimerization power of sulphur in this case proved to be modest (*ca*.10%) in response to probably less clear-cut kinetic and thermodynamic criteria Indeed the attacked 2α C-H bond is not as well exposed as the 17β C-H bond, and the sterical decompression resulting from the 2β-methyl/2α-methyl conversion is probably not so important, as the ring-A may adopt a twisted conformation to minimize the steric interactions of its 2β-methyl group with the neighbouring 4β- and 10β-methyl groups [15] The mere existence of this isomerization could even be questioned as the enhancement in 2α-methyl isomer after the heating experiments could only reflect a greater tendency of the 2β-methyl isomer to decompose to more polar compounds (see Table 1) The fact, however, that the 2α-methyl/2β-methyl isomeric ratio does not increase on prolonged heating at 240°C (Table 1, entry 3) precludes this possibility In the case of hopanoid **1a**, the slowness of the 2β-methyl/2α-methyl epimerization compared to the 17β,21β/17α,21β and to a lesser extent 17β,21β/17β,21α ones is particularly enlightened as hopanoid composition after the heating experiment essentially reflects the epi-

merization at C-17 Two features have to be kept in mind. The reaction conditions we used (molten sulphur) are less likely to occur in sediments and the apparent isomerization we observed might be the result of a more complex process involving the formation of functionalized intermediates and their decomposition into the detected end products [16]

Our results suggest a dual origin for the presence of 2α-methylhopanoids in sediments *i e* the direct incorporation of prokaryotic 2α-methylhopanoids and/or abiotic isomerization of 2β-methyl isomers However, several questions remain, considering the reported predominence of sedimentary 2α-methyl isomers on the one hand, and the low epimerization power of sulphur on the other hand Do 2α-methylhopanoids have a broad distribution in prokaryotes? Does diagenesis involve other non sulphur-induced epimerization processes? Could it be only that the presence of 2β-methyl and 2α-methylhopanoids in sediments has been much overlooked? Indeed, in sedimentary mixtures of methylated and non-methylated hopanoids, detection of 2β-methylhopanoids, especially in small amounts, appears delicate as, contrary to their 2α and 3β isomers, they possess gc retention times comparable to those of non-methylated hopanoids

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EXPERIMENTAL

General

Most of the analytical procedures and separation schemes were as described previously [1a] Gc analyses were performed on a CARLO ERBA Fractovap 4160 chromatograph equipped with a flame ionization detector fitted with a DB-1 surface-bonded phase fused silica capillary column (30m, film thickness=0 1μm) using a detector temperature of 310°C and a standard temperature program from 50°C to 220°C (20°C/min) and from 220°C to 310°C (4°C/min) with a hydrogen pressure of 0 7kg cm^{-2} When required (Table 1), nearly base line separation between 2α-methyl and 2β- methylhopanes isomers was obtained using a slower temperature program *i e* from 50°C to 175°C at 20°C/min and from 175°C to 310°C at 0.5°C/min Electronic impact gc/ms analyses were performed at 70eV on a LKB 9000S spectrometer as previously reported[17] or on a KRATOS MS80RF mass spectrometer with a source temperature of 280°C and a DB-5 (30m x 0.25mm) capillary column. ^{1}H-nmr and ^{13}C-nmr spectra were recorded in $C^{2}HCl_3$ on BRUKER W200 or W400 or ACF250 apparatuses using $CHCl_3$ (δ=7.260ppm) as internal standard for ^{1}H-nmr or $^{13}C^{2}HCl_3$ (δ=77 03ppm) for ^{13}C-nmr. Melting points were measured after recrystallization from CH_2Cl_2/CH_3OH using a REICHERT-JUNG micro hot-stage and are uncorrected.

3,22-Bistrimethylsiloxy-hop-2-ene 9

To a solution of hydroxyhopanone **8a** (105mg) in DMF (2ml) and triethylamine (1ml), chlorotrimethylsilane (1ml) was added. The resulting mixture from which a pale yellow solid (presumably triethylamine hydrochloride) separated immediately was heated for 15h at 50°C. After cooling, dilution with ether (10ml) and washing with aq $NaHCO_3$ (10ml), the organic layer was dried (Na_2SO_4) and concentrated, yielding the required bistrimethylsilyl ether **9** (125mg) next to 22-trimethylsiloxy-hopan-3-one **8b** (10mg) after flash chromatography (Si-gel, 5% EtOAc-cyclohexane)

9, ^{1}H-nmr (200MHz), δ 0.0094 (9H, s), 0 179 (9H, s), 0 737 (3H, s), 0 848 (3H, s), 0 940 (3H, s), 0 971 (3H, s), 1 000 (3H, s), 1 146 (3H, s), 1 245 (3H, s), 1 962 (1H, dd, J=16 & 6 6Hz), 2 000 (2H, m), 4.573 (1H, dd, J=1 8 & 6 6Hz) ppm

8b, m p =194-195°C, ^{1}H-nmr (200MHz); δ 0 093 (9H, s), 0.735 (3H, s), 0 919 (3H, s), 0.942 (3H, s), 0.990 (3H, s), 1.020 (3H, s), 1 073 (3H, s), 1.144 (3H, s), 1 245 (3H, s), 2 382 (1H, ddd, J=4.5 & 7 5 & 15 5Hz), 2 507 (1H, ddd, J=7 & 9 5 & 15 5Hz) ppm.

2α- and 2β-Methylhopan-22-ol-3-one 10b and 10a

Benzyltrimethylammonium fluoride (105mg) was dried overnight in THF (2ml) in the presence of 4Å molecular sieve (600mg) under an argon atmosphere To this suspension, a solution of bistrimethylsilyl ether **9** (50mg) in THF (2ml) and CH_3I (30μl) were added. After stirring of the mixture for 1h at room temperature and 4h at 50°C, filtration and removal of the solvent, tlc purification (Si-gel, 5% EtOAc-toluene), next to unreacted starting compound **9** (R_f=0.82, 4mg) and trimethylsilyl ether **8 b** (R_f=0 41, 15mg, 34%), yielded 22-*O*-trimethylsilylether of 2-methyl-22-hydroxyhopan-3-one (R_f=0.53, 21mg) A solution of the latter (18mg) in MeOH (1ml) and THF (1ml) was stirred, after addition of HCl 6N (15μl), for 2h at room temperature Removal of the solvent and tlc purification (Si-gel, 5% EtOAc-toluene), besides unreacted starting product (1 4mg), afforded 2-methylhydroxyhopanone **10a** and **10b** (15 mg) which appeared to be a 4 1 mixture of 2α 2β methyl isomers (^{13}C-nmr) The 2α- and 2β-methylhydroxyhopanone **10b** and **10a** were obtained pure after C_{18} reverse phase hplc purification using MeOH H_2O, 92 8, v v as eluent

10a, ^{1}H-nmr (250MHz), δ 0 655 (3H, s), 0 776 (3H, s), 0.944 (3H, s), 0 975 (3H, d, J=6 4Hz), 0 988 (3H, s), 1 023 (3H, s), 1 056 (3H, s), 1 180 (3H, s), 1 210 (3H, s), 2 232 (1H, m), 2 804 (1H, ddq, J=6 4 & 9 4 & 11 0Hz) ppm

10b, ^{1}H-nmr (250MHz), δ 0.759 (3H, s), 0.929 (3H, s), 1 016 (3H, d, J=6.4Hz), 1 018 (3H, s) 1 036 (3H, s), 1 062 (3H, s), 1 106 (3H, s), 1.180 (3H, s), 1 211 (3H, s), 2 229 (1H, m), 2 752 (1H, quintuplet, J=6 4Hz) ppm

2α-Methyldiplopterol 4 b

2α-Methylhydroxyhopanone **10b** was converted to 2α-methyldiplopterol **4b** after Wolff-Kishner reduction as already described [12]

4b m p =194-196°C, ^{1}H-nmr (200MHz) see ref.3a, ^{13}C-nmr (63MHz), δ 16 14 (C-28), 16 65 (C-25), 16 74 (C-27), 17.05 (C-26), 18 61 (C-6), 20 91 (C-11), 21.95 (C-16), 22.22 (C-24), 23 15 (C-31), 23 90 (C-2), 24 11 (C-12), 26.62 (C-20), 28 72 (C-29), 30 86 (C-30), 33 25 (C-7), 33 47 (C-23), 33 94 (C-4), 34 38 (C-15), 38 05 (C-10), 41 22 (C-19), 41 84 (C-14), 41 90 (C-8), 44 09 (C-18), 49.57 (C-3), 49 81 (C-13), 50 33 (C-9), 51 11 (C-21), 51 24 (C-1), 53.90 (C-17), 55 74 (C-5), 73 99 (C-22) ppm

2β-Methyl-17β-hop-21-ene 5a and 2β-methyl-17β-hop-22(29)-ene 7a

To a solution of tertiary alcohol **4a** (20mg) in a 2:1 mixture of CH_2Cl_2/pyridine (1 5ml) was added at 0°C under stirring freshly distilled thionyl chloride (100µl). After 15min at 0°C, the medium was quenched over aq. Na_2CO_3 and diluted with pet ether The organic layer was dried (Na_2SO_4), evaporated and purified by tlc (Si-gel, 10% $AgNO_3$, pet ether-toluene, 95:5) to give **5a** (R_f=0.45, 6mg), **7a** (R_f=0 25, 8mg) next to traces of **6a** (R_f=0.35, 0 3mg)

5a, m.p =173-174°C, ^{1}H-nmr (400MHz), δ 0.599 (3H, d, J=0 7Hz), 0.840 (3H, d, J=6 3Hz), 0.845 (3H, s), 0.900 (3H, s), 0 909 (3H, s), 0 968 (6H, s), 1.589 (3H, s), 1 741 (3H, s), 2 2 (3H, s) ppm

ms m/z 424 (M^+, 78%), 409 (18%), 381 (52%), 355 (64%), 313 (8%), 245 (22%), 219 (10%), 217 (11%), 205 (82%), 189 (100%), 177 (18%), 175 (22%), 161 (51%).

7a, m p.=202-203°C; ^{1}H-nmr (400MHz); δ 0 732 (3H,s), 0.833 (3H, d, J=7Hz), 0.838 (3H, s), 0.891 (3H, s), 0.900 (3H, s), 0 943 (3H, s), 0 957 (3H, s), 1 758 (3H, s), 2.69 (1H, dd, J=7 & 9Hz), 4.788 (2H, s) ppm

ms m/z 424 (M^+, 32%), 409 (10%), 315 (6%), 313 (8%), 218 (10%), 205 (80%), 189 (100%), 177 (8%), 175 (7%), 163 (7%), 161 (16%)

2α-Methyl-17β-hop-21-ene 5b and 2α-methyl-17β-hop-22(29)-ene 7b

Preparation of the two methylhopenes **5b** and **7b** was realized as described for **5a** and **7a** (see above)

5b, m.p =169-170°C, ^{1}H-nmr (400MHz); δ 0.583 (3H, d, J=0,8Hz), 0.800 (3H, s), 0.827 (3H, s), 0 829 (3H, d, J=6.2Hz), 0.852 (3H, s), 0.959 (3H, s), 0.965 (3H, s), 1 577 (3H, s), 1 730 (3H, s) ppm

ms m/z 424 (M^+, 35%), 409 (8%), 381 (30%), 355 (36%), 245 (27%), 205 (100%), 189 (83%), 161 (62%).

7b, m p =205-206°C; ^{1}H-nmr (400MHz); δ 0.716 (3H, d, J=1Hz), 0.792 (3H, s), 0 818 (3H, s), 0 823 (3H, d, J=6 2Hz), 0 843 (3H, s), 0 932 (3H, s), 0 955 (3H, s), 1 748 (3H, s), 2 677 (1H, dt, J=7 & 8Hz), 4.77 (2H, s) ppm

ms m/z 424 (M^+, 14%), 409 (6%), 381 (4%), 205 (100%), 189 (99%), 177 (7%), 175 (9%), 163 (10%), 161 (20%)

2β-Methylhop-17(21)-ene 6a from 4a

After dissolution of 2β-methyldiplopterol **4a** (15mg) in a minimum amount of $CHCl_3$ (*ca* 0 3ml), HCO_2H (0 3ml) and 70% $HClO_4$ (50µl) were added under stirring The biphasic medium was stirred at room temperature vigorously for 10min, quenched over aq $NaHCO_3$ and diluted with pet ether After drying (Na_2SO_4), evaporation of the solvents and tlc purification (Si-gel, 10% $AgNO_3$, 5% toluene-pet ether), **6a** (R_f=0 35, 13 5mg) was obtained as a colourless solid

6a, m p =138-139°C, ^{1}H-nmr (400MHz), δ 0 838 (3H, s), 0 84 (3H, d, J=6.5Hz), 0.847 (3H, s), 0.898 (3H, s), 0 916 (3H, s), 0 922 (3H, s), 0 925 (3H, d, J=6 7Hz), 0 984 (3H, d, J=6 8Hz), 1 041 (3H, s), 1 92 (1H, m), 2 11 (1H, dd, J=9 & 15Hz), 2 26 (1H, dt, J=6 5 & 15Hz), 2 65 (1H, sept , J=7Hz) ppm

ms m/z 424 (M^+, 57%), 409 (18%), 381 (100%), 245 (42%), 205 (37%), 203 (12%), 189 (24%), 175 (24%), 161 (38%)

2α-Methylhop-17(21)-ene 6b

Methylhopene **6b** was prepared from **5b** as previously reported on its non-methylated homologue [7]

6b, m p =147-149°C; ^{1}H-nmr (400MHz), δ 0 795 (3H, d, J=6Hz), 0 833 (6H, s and 3H, d, J=6Hz), 0 851 (3H, s), 0.914 (3H, d, J=6.9Hz), 0 923 (3H, s), 0 974 (3H, d, J=6 9Hz), 1 034 (3H, s), 2 2 (2H, m), 2 64 (1H, sept, J=6 9Hz) ppm.

ms m/z 424 (M^+, 22%), 409 (13%), 381 (79%), 245 (64%), 205 (65%), 189 (34%), 180 (45%), 161 (75%), 135 (100%)

2α-and 2β-Methyl-17β-hopanes 1b and 1a

Both methylhopanes **1a** and **1b** were prepared by hydrogenation of **7a** and **7b** as previously described [8]

1a, m p =195-196°C; ^{1}H-nmr (400MHz), δ 0 702 (3H, s), 0.805 (3H, d, J=6 2Hz), 0 824 (3H, d, J=6 4Hz), 0 826 (3H, s), 0 881 (3H, d, J=6 2Hz), 0 824 (3H, d, J=6 4Hz), 0 826 (3H, s), 0 881 (3H, s), 0 889 (3H, s), 0 926 (3H, d, J=7Hz), 0 935 (6H, s) ppm

ms m/z 426 (M^+, 3%), 411 (4%), 383 (6%), 205 (42%), 191 (100%), 163 (12%)

1b, m p =213-214°C, ^{1}H-nmr (400MHz), δ 0 694 (3H, s), 0 791 (3H, s), 0 799 (3H, d, J=6 5Hz), 0 815 (3H, s), 0 822 (3H, d, J=6 5Hz), 0 844 (3H, s), 0 924 (3H, d, J=6 5Hz), 0 934 (3H, s), 0 943 (3H, s) ppm

ms. m/z 426 (M^+, 4%), 411 (4%), 383 (6%), 205 (48%), 191 (100%), 163 (10%)

2β-Methyl-17α,21β-hopane 2a

After dissolution of the tetrasubstituted olefin **6a** (10mg) in a 1 1 mixture of AcOEt AcOH (6ml), 70% $HClO_4$ was added (0.15ml) followed by palladium hydroxide (30mg) After 20h of stirring at 20°C under hydrogen (1atm), the mixture was quenched over aq $NaHCO_3$, diluted with pet ether, dried (Na_2SO_4) and evaporated to dryness giving **2a** (R_f=0 85, 6mg) as a colourless solid after tlc purification (Si-gel, 10% $AgNO_3$; pentane)

2a, m p =138-139°C, ^{1}H-nmr (250MHz), δ 0 815 (3H, d, J=6.5Hz), 0 824 (3H, s), 0 832 (3H, d, J=6 5Hz), 0 840 (3H, s), 0 892 (3H, s), 0 895 (3H, d, J=6 5Hz), 0 907 (3H, s), 0 925 (3H, s), 0.946 (3H, s), 0.995 (3H, s) ppm.

ms m/z 426 (M^+, 8%), 411 (9%), 383 (2%), 231 (4%), 218 (11%), 205 (100%), 191 (48%), 177 (8%), 163 (22%).

2α-Methyl-17α,21β-hopane 2b

Methylhopane **2b** was prepared from **5b** as already described for the non-methylated series [7,11]

2b, m p =88-89°C, ^{1}H-nmr (400MHz); δ, 0 798 (3H, s), 0.806 (3H, d, J=6 5Hz), 0 829 (3H, s), 0 829 (3H, d, J=6Hz), 0 848 (3H, s), 0 893 (3H, d, J=6 5Hz), 0.956 (3H, s), 0 992 (3H, s) ppm

ms m/z 426 (M^+, 8%), 411 (9%), 383 (2%), 231 (4%), 218 (11%), 205 (100%), 191 (48%), 177 (8%), 163 (22%)

2α-and 2β-Methyldiplopterols 4b and 4a from Methylobacterium organophilum

2α-Methyldiplopterol **4b** was identified in trace amount (5%) in the ^{13}C-nmr spectrum of a sample of 2β-methyldiplopterol (20mg) previously isolated from *M organophilum*[3a] by comparison with the spectrum of synthetic 2α-methyldiplopterol of reference

2-Methyldiplopterols from *M. organophilum*

^{13}C-nmr (63MHz), δ 16 14 (**4b**, C-28), 16.28 (**4a**, C-27 and C-28), 16.64 (**4b**, C-25), 16 74 (**4b**, C-27), 16 94 (**4a**, C-26), 17 04 (**4b**, C-26), 18 61 (**4b**, C-6), 19 91 (**4a**, C-6), 20 89 (**4b**, C-11), 21 75 (**4a**, C-25), 21 86 (**4a**, C-11), 21.94 (**4a** and **4b**, C-16), 22 21 (**4b**, C-24), 23 15 (**4b**, C-31), 23 21 (**4a**, C-31), 23 89 (**4b**, C-2), 24 10 (**4b**, C-12), 24 42 (**4a**, C-20), 26 61 (**4b**, C-20), 28.70 (**4a** and **4b**, C-29), 30 84 (**4a** and **4b**, C-30), 31 03 (**4a**, C-23), 32 44 (**4a**, C-4), 32.47 (**4a**, C-7), 33.24 (**4b**, C-7), 33 46 (**4b**, C-23), 33 93 (**4b**, C-4), 34 36 (**4b**, C-15), 34 40 (**4a**, C-15), 37 78 (**4a**, C-10), 38 04 (**4b**, C-10), 41 21 (**4b**, C-19), 41 29 (**4a**, C-19), 41.82 (**4b**, C-14), 41 88 (**4a**, C-14 and **4b**, C-8), 42 00 (**4a**, C-8), 44 08 (**4b**, C-18), 44 10 (**4a**, C-18), 45 15 (**4a**, C-1), 49 56 (**4a** and **4b**, C-13), 49 70 (**4a**, C-3), 49 80 (**4b**, C-13), 50 27 (**4a**, C-9), 50 31 (**4b**, C-9), 51.10 (**4a** and **4b**, C-21), 51 23 (**4b**, C-1), 53 88 (**4b**, C-17), 53 92 (**4a**, C-17), 55 72 (**4b**, C-5), 73 99 (**4a** and **4b**, C-22) ppm

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