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Novel Hopanoid Derivatives Released by Oxidation of Messel Shale Kerogen

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Abstract: *Oxidation of Messel shale kerogen using ruthenium tetroxide yielded several series of hopanoid triterpenes which have been identified by synthesis. These compounds could originate from hopenes or hopanes of bacterial origin linked to the kerogen; their structures indicate that incorporation processes into macromolecular entities of their biological precursors must occur at a rather early stage of diagenesis.* © 1997 Elsevier Science Ltd.

INTRODUCTION

Up to 90% of the geological organic matter occurs in sediments and soils as macromolecular material (kerogen, humin) insoluble in organic solvents. Kerogen is thought to result from the condensation of biolipids and selective preservation of biopolymers[1] originating from decaying organisms. Due to its structural complexity, several methods have been developed to investigate the nature and mode of reticulation of the sub-units present in the kerogen[2] and among them, chemical degradations which cleave selectively, for example, ester, C-S, C-O or C-C bonds. In this respect, ruthenium tetroxide (RuO_4) is a useful oxidizing agent since it degrades aromatic units and liberates their alkyl substituents as carboxylic acids bearing one extra carbon coming from the aromatic ring.[3-7] Other functionalities may, however, also be altered in this process.[8,9] In this study, RuO_4 oxidation has been employed to investigate the nature of the sub-units present in the Eocene Messel shale kerogen (near Darmstadt, Germany) and to provide additional information to that already obtained previously by several authors using pyrolysis[10,11] or chemical degradations.[6,12-19]

We report here the characterisation by synthesis of several oxidation products from the kerogen related to the hopane triterpene series. Their precise identification together with the selectivity of RuO_4 enable us to propose modes of incorporation of these hopanoids of bacterial origin within the macromolecular network of the kerogen.

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RESULTS AND DISCUSSION

The Messel kerogen obtained by HF/HCl digestion of the pre-extracted sediment[20] was treated with ruthenium tetroxide under catalytic conditions.[21] The crude mixture obtained by extraction was esterified with diazomethane and submitted to liquid chromatography over silica gel which gave three fractions of increasing polarity. Only the less polar fraction eluted with ether (3.8% of the starting kerogen) was considered; it was further fractionated by silica gel thin layer chromatography (TLC) eluting with dichloromethane into two sub-fractions (R_F=1 - 0.5 and R_F=0.5 - 0.1). Gas chromatography-mass spectrometry (GC-MS) analysis of these two TLC fractions showed the predominance of two series of aliphatic mono- and α,ω-diacids (as methyl esters) which reflect the high degree of aliphaticity of the Messel shale kerogen. Saturated hopanoic acids mainly belonging to the (17βH, 21βH) series and ranging from C_{30} up to C_{32} were also present in the monoacid fraction. Benzene di- to hexa-carboxylic acids, in trace amounts, result from the oxidation of polyaromatic sub-units occurring in the kerogen structure. In addition, a series of unknown compounds displaying the polarity of diesters was also detected. Their molecular ions and an intense *m/z* 191 fragment in their mass spectra indicated that they could correspond to hopanoic triterpene acids bearing one or two additional functionalities and ranging from C_{26} to C_{32}. Detailed analysis of various mass fragments was indeed in agreement with this assumption and allowed to establish several structural hypotheses.

Significantly diagnostic were fragments *m/z* 231 and *m/z* 367 displayed by compound **1** (figure 1) which were consistent with a C_{32} pentacyclic hopane acid ester (M^+=482) bearing a double bond in the 17(21) position.[22] In comparison with **1**, compound **2** showed an upward shift of 16 a.m.u in the molecular ion (M^+=498) and in the side chain cleavage (*m/z* 383) which is compatible with the presence of an epoxide function at position 17(21). Ring E degraded tetracyclic hopanoic acid esters presumably resulting from the oxidative cleavage of 17(21) double bond seemed to be present as major components, as indicated by the occurrence of fragments at either *m/z* 343 or *m/z* 344 from compounds **3** to **6** (M^+=416, 430, 500 and 514 respectively). Whereas fragment *m/z* 343 is interpretable in terms of the mass loss of a functionalized side-chain, the even fragment *m/z* 344 observed in higher members **5** and **6** could result from a McLafferty rearrangement due to the presence of the ketone function at C-17.

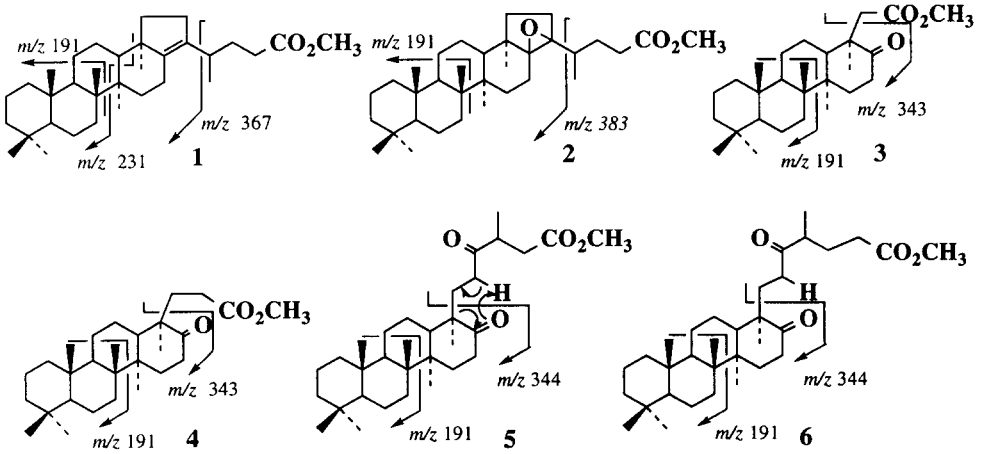
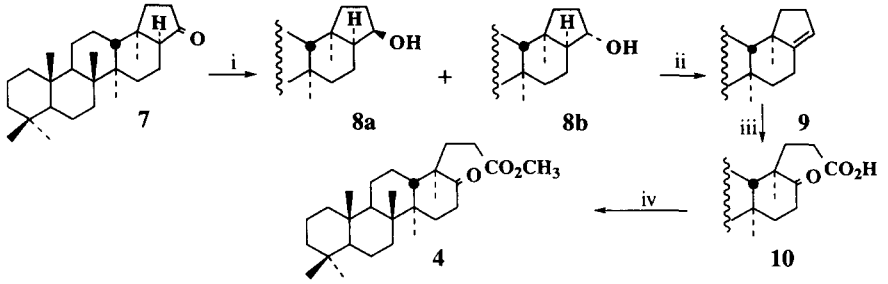


Figure 1: Characteristic mass fragments of some hopanoid derivatives detected in the oxidation products of the Messel shale kerogen

Synthesis of compounds **1**, **2**, **4** and **6** has been carried out on in order to confirm these structural hypotheses.

Synthesis of 22,29,30-trinor-17,21-secohopan-17-on-21-oic acid, methyl ester 4

The synthesis of compound **4** is outlined in scheme 1.



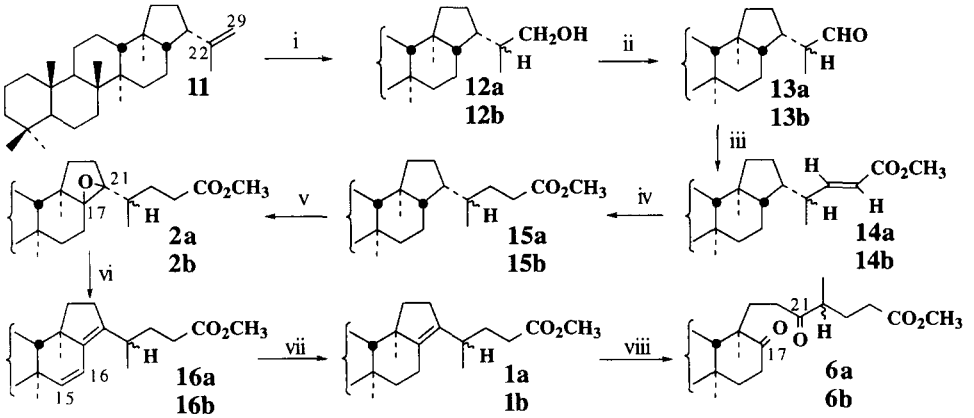
i: $LiAlH_4$, THF; ii: $POCl_3$, pyridine; iii: RuO_2, $NaIO_4$, acetone, H_2O; iv: CH_2N_2

Scheme 1: Synthesis of 22,29,30-trinor-17,21-secohopan-17-on-21-oic acid, methyl ester **4**

The starting material, 22,29,30-trinor-17α-hopan-21-one **7**, was obtained from 22-hydroxyhopan-3-one as described previously.[23] Lithium aluminium hydride reduction of ketone **7** gave a mixture (80:20) of 22,29,30-trinor-17α-hopan-21β-ol and 21α-ol, **8a** and **8b**, readily separable by TLC. Treatment of the alcohol mixture with phosphorus oxychloride/pyridine furnished alkene **9** which upon oxidation with ruthenium dioxide and sodium periodate in aqueous acetone solution[24] afforded the ketoacid **10,** which was further esterified with diazomethane[25] to yield methyl ester **4**.

Synthesis of the C_{32} hopanoids 1, 2 and 6

The synthetic scheme used for preparation of the C_{32} reference compounds (**1**,**2** and **6**) is shown in scheme 2.



i: B_2H_6, THF; H_2O_2 30%, NaOH 3M; ii: PDC, CH_2Cl_2; iii: $Ph_3P{=}CHCO_2CH_3$; iv: H_2, PtO_2, hexane, diethylether; v: *m*CPBA, $CHCl_3$; vi: PPh_3, I_2, CH_2Cl_2; vii: H_2, Pd/C, AcOEt; vii: RuO_4, CCl_4

Scheme 2: Synthetic scheme for the preparation of the C_{32} hopanoids **1**, **2** and **6**

Hydroboration-oxidation of diploptene **11** followed by oxidation of **12** with pyridinium dichromate[26] gave **13a** and **13b**, epimers at C-22. Intermediates **14a** and **14b** were obtained from the mixture of the two aldehydes using a Wittig reaction.[27] Their catalytic hydrogenation with PtO_2 furnished the methyl esters **15a** and **15b.** The regioselective oxidation of **15** at unactivated positions 17 and 21 with mCPBA[28] afforded the 17,21-epoxides **2**, not separable by TLC and GC. Deoxygenation of **2** using triphenylphosphine-iodine complex[29] yielded the dienes **16** which were selectively converted by catalytic hydrogenation into monoenes **1a** and **1b** readily separable on silica gel impregnated with silver nitrate. The two isomers **6a** and **6b** were obtained by ruthenium tetroxide oxidation of the olefin mixture **1**.

The identification of the oxidation products of the Messel kerogen was clearly established by comparison with synthetic components **1-3** and **6** on the basis of nearly superimposable mass spectra and identical GC elution time (co-injections on two different columns). The structures of the lower homologues **3** and **5** were inferred from their mass spectra (*m/z* 343 and 344 in compounds **3** and **5** respectively).

The presence of hopanoid derivatives linked to macromolecular organic matter such as kerogens or asphaltenes *via* sulfide, ether, ester or carbon-carbon bridges has been demonstrated by several authors.[9,30,31] In particular, Trifilieff *et al.* have shown that hopanoic acids can be released by treating asphaltenes with ruthenium tetroxide.[4] These authors have suggested that sensitive biological hopanoid precursors such as hopanepolyols, which are widespread bacterial membrane constituents[32], or their dehydration products (hopanepolyenes) could become covalently linked to aromatic subunits present in the macromolecular network by an acid catalysed process occurring during early stages of the diagenetic decay of organic matter in the sediment (figure 2). The identification of compounds **1**, **2**, **5** and **6** in the oxidation products of the Messel kerogen, essentially as one epimer at C-22, suggests at least two possible explanations given the fact that up to now no hopanoid polyols bearing an unsaturation at C-17 have been detected in living organisms. First, the corresponding 17(21)-enes could be formed from the polyols by oxidation followed by dehydration during early diagenesis in the sediment or even the water column, since it is known that both positions are sensitive to oxidizing reagents.[28] An alternative explanation could be the oxidation of these positions by ruthenium tetroxide, a possibility which we have checked on C_{30} (17βH, 21βH)-hopane. Under the conditions used the latter did indeed yield about 10% of a mixture of hop-17(21)-ene, 17(21) epoxide and the diketone corresponding to the cleavage of the 17(21) double bond. The distribution of the (17βH, 21βH) hopanoic acids obtained by oxidative cleavage, largely dominated by the C_{30} homolog, appears, however, rather in disfavor of this second hypothesis. It is noteworthy that the more stable C_{35} (17αH, 21βH) hopane remained unaffected by the reagent.

Minor compounds **1** and **2** could then correspond to uncomplete oxidation of the bound or neoformed hop-17(21)-enes under the conditions used. It is interesting to notice that the occurrence of bound hop-17(21)-enes in the kerogen of the Messel shale goes along with the predominance of free hop-17(21)-enes in the organic extract.

Compound **3** could be obtained by oxidation of a bound C_{32} benzohopane.[33] Benzohopanes occur in the organic extract and hence could exist in a bound form. Indeed, the treatment with RuO_4 of a fraction from a sediment extract highly enriched in benzohopanes yielded the oxidation compound **3** suggesting that this precursor can be envisaged.

Lastly, oxidation product **4** could be interpreted as resulting from the oxidation of a C_{27} hopan-21-one or 21-ol attached to the macromolecular matrix *via* an ether linkage.

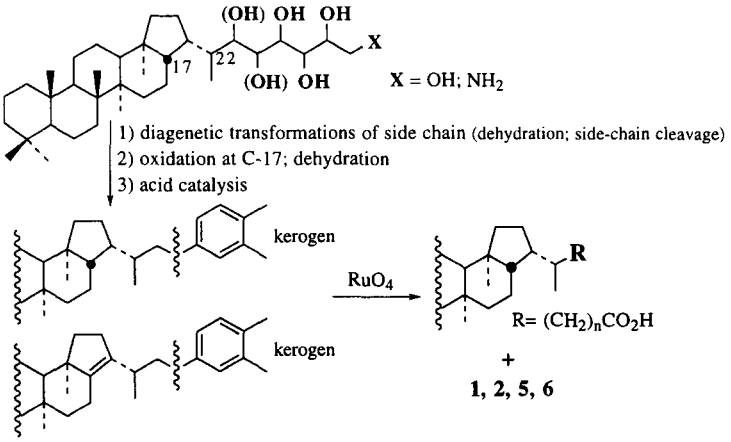


Figure 2: Possible origin of oxidation products **1**, **2**, **5**, **6** and hopanoic acids from hopanoid moieties bound to aromatic subunits in kerogen, presumably formed during diagenetic decay of polar hopanoids derived from bacteria.

EXPERIMENTAL

Extraction procedure: The powdered sediment was soxhlet-extracted with a mixture of toluene/MeOH 3:1 v/v (4 x) and demineralised with a mixture of HCl/HF 1:1 v/v (RT, 24h). The solid residue obtained after filtration, was washed with a solution of HCl 3N and then with water until a neutral pH was obtained. The residue recovered after centrifuging was extracted with acetone and mixtures of $CHCl_3$/MeOH 3:1 v/v, 2:1 and 1:1. After a final soxhlet-extraction ($CHCl_3$, 24h), the crude kerogen was dried under vacuum. **Ruthenium tetroxide oxidation**[21]: 10 ml of CCl_4, 20 ml of distilled water, 10 ml of CH_3CN and 5 g of sodium periodate were added to 500 mg of kerogen and stirred for 30 min at RT. 50 mg of RuO_2 were added to generate *in situ* RuO_4 and the mixture was maintained under stirring for 4 h. The crude was filtered and the residue was washed with CH_2Cl_2 and distilled water. After extraction with CH_2Cl_2 and diethyl ether, the organic extracts were combined and the solvent removed under reduced pressure. The extract was esterified with diazomethane and separated into three fractions by column chromatography over silica gel eluting respectively with ether, ether/MeOH 1:1 v/v and $CHCl_3$/MeOH/H_2O 65:25:4 v/v/v. The first-eluting fraction was further subjected to preparative TLC (silica gel, CH_2Cl_2 as developer) affording two sub-fractions (R_F=1-0.5 containing the monoesters and R_F=0.5-0.1 containing the diesters). **Gas Chromatography** (GC): Analyses were performed on a Carlo Erba Fractovap 4160 chromatograph with "on-column" injector and FID, fitted with a J&W DB5 capillary column (30 m x 0.3 mm, film thickness: 0.25 μm). **Gas Chromatography-Mass Spectrometry** (GC-MS): GC-MS analyses were accomplished on a Varian 3400 gas chromatograph fitted with a temperature programmable on-column injector and a DB5 column (30 m x 0.25 mm, 0.1 μm film thickness), and linked to a Finnigan MAT INCOS 50 mass spectrometer (EI, 70 eV). Co-injections were performed on a Varian 3400 chromatograph equipped with an "on-column" injector connected to a Finnigan MAT TSQ 70 mass spectrometer operating at 70eV. **Direct Insertion probe MS** (DI): DI analyses were carried out using a Finnigan MAT TSQ 70 mass spectrometer (EI, 70eV). **Nuclear Magnetic Resonance**: ^{1}H NMR spectra were obtained either on Bruker AM-400 or WP-200 SY spectrometers. **Infrared Spectra** (IR): Spectra were recorded on a Perkin-Elmer FT-IR 1600 spectrophotometer.

22,29,30-Trinor-17α-hopan-21-ol 8a (21β) and **8b** (21α): To 70 mg of lithium aluminium hydride in THF were added 330 mg (0.9 mmol) of 22,29,30-trinor-17α-hopan-21-one **7** in 40 ml of anhydrous THF. The reaction mixture was stirred under argon for 3 h and the excess of $LiAlH_4$ was destroyed with cold water. The product was extracted with ether, washed with a saturated $NaHCO_3$ solution and dried over magnesium sulfate to afford 317 mg (96%) of the two alcohols **8a** and **8b** (80:20). A fraction from the mixture was purified by TLC (CH_2Cl_2). **8a**: R_F=0.30 (CH_2Cl_2); Anal. calcd. for $C_{27}H_{46}O_1$: C, 83.87; H, 11.99. Found: C, 84.03; H, 12.10; mp 201-210°C; ^{1}H NMR (400MHz, C_6D_6) δ (ppm) 0.67 (d, J = 4Hz, 1H, OH-21), 0.86, 0.87, 0.91, 0.97, 1.05, 1.06 (6s, 18H, quaternary CH_3), 1.96-2.03 (m, 1H), 3.98-4.03 (m, 1H, H-21); MS (DI, 70eV), *m/z* (rel. int.) 386(M^+, 27%), 368(9), 353(8), 191(100), 177(8), 162(37), 147(29). **8b**: R_F=0.15 (CH_2Cl_2); ^{1}H NMR (400MHz, C_6D_6) δ (ppm) 0.80 (d, J = 5.6Hz, 1H, OH-21), 0.84, 0.85, 0.87, 0.90, 0.97, 0.98 (6s, 18H, quaternary CH_3), 1.91-1.98 (m, 1H), 4.06 (m, 1H, H-21); MS (DI, 70eV), *m/z* (rel. int.) 386(M^+, 20%), 371(5), 353(3), 191(100), 177(7), 165(10), 147(8).

22,29,30-Trinorhop-17(21)-ene 9: 300 mg (0.78 mmol) of a mixture of the two alcohols **8a** and **8b** dissolved in 40 ml of anhydrous pyridine were treated by 140 μl of phosphorus oxychloride. The reaction was stirred for 6 h at RT under argon and quenched with a cold saturated $NaHCO_3$ aqueous solution. The product was diluted with ether, washed with water and dried over $MgSO_4$. The resulting residue was then purified by silica gel chromatography eluting with hexane to obtain 245 mg of the olefin **9** (82%). R_F=0.85 (hexane); ^{1}H NMR (400MHz, $CDCl_3$) δ (ppm) 0.79, 0.83, 0.85, 0.89, 0.94, 1.05 (6s, 18H, quaternary CH_3), 2.07-2.30 (m, 4H, H-16 and H-20), 5.15 (m, 1H, H-21); MS (GC, 70eV), *m/z* (rel. int.) 368(M^+, 15%), 353(7), 231 (86), 191(100), 177(10), 161(43), 147(46).

22,29,30-Trinor-17,21-secohopan-17-on-21-oic acid 10: A solution of the alkene **9** (200 mg) in acetone (100 ml) was added dropwise to a ruthenium tetroxide solution prepared by stirring 72 mg of ruthenium dioxide in 50 ml of acetone with 400 mg of sodium periodate in a minimum amount of water. The mixture was

stirred for 3 h and during this time portions of a sodium periodate solution, prepared by dissolving 500 mg of $NaIO_4$ in water, were added. The excess of RuO_4 was destroyed by addition of 10 ml of isopropyl alcohol. The crude was filtered over celite and the solvent removed *in vacuo*. The product was diluted with CH_2Cl_2, dried over $MgSO_4$ and purified by preparative TLC (hexane/ethyl acetate, 1:1) to afford 94 mg (45 % yield) of compound **10**. R_F=0.40 (hexane/ethyl acetate 1:1 v/v); ^1H NMR (200MHz, $CDCl_3$) δ (ppm) 0.80, 0.84, 0.85, 1.00, 1.03, 1.07 (6s, 18H, quaternary CH_3), 2.18-2.31 (m, 2H, H-16 or H-20), 2.34-2.48 (m, 2H, H-16 or H-20); MS (DI, 70eV), *m/z* (rel. int.) 416(M^+, 5%), 398(5), 383(3), 344(3), 343(3), 231(4), 191(100), 177(11), 164 (10), 149(10).

22,29,30-Trinor-17,21-secohopan-17-one-21-oic acid, methyl ester 4: A fraction of acid **10** solubilised in ether was esterified with diazomethane, affording quantitatively the methyl ester **4**. R_F=0.30 (CH_2Cl_2); ^1H NMR (400MHz, $CDCl_3$) δ (ppm) 0.80, 0.84, 0.85, 1.00, 1.02, 1.07 (6s, 18H, quaternary CH_3), 1.85-2.03 (m, 3H), 2.13-2.29 (m, 2H, H-16 or H-20), 2.33-2.49 (m, 2H, H-16 or H-20), 3.65 (s, 3H, -OC<u>H</u>$_3$); GC-MS (70eV), *m/z* (rel. int.) 430(M^+, 14%), 415(4), 343 (4), 191(100), 177(11), 163(9); IR ($CHCl_3$) 1387-1458, 1697, 1730 cm^{-1}.

Hopan-29-ol 12a and **12b**: 8 ml of a solution 1M of B_2H_6 (8 mmol) in THF were added to 200 mg (0.49 mmol) of hop-22(29)-ene **11** dissolved in 50 ml of anhydrous THF.[34] The mixture was stirred under argon for 7 hours, after which 10 ml of 3M NaOH and 20 ml of H_2O_2 30% were added. After stirring overnight, the reaction mixture was extracted with ether, dried over Na_2SO_4 and the solvent removed under reduced pressure. Silica gel chromatography followed by a recrystallization in methylene chloride/diisopropyl ether afforded 148 mg (72% yield) of a mixture of the two epimers in position 22 **12a** and **12b**, which were not separable by liquid chromatography or GC, but could be distinguished by NMR, in a ratio 70:30. R_F=0.25 (CH_2Cl_2); Anal. calcd. for $C_{30}H_{52}O_1$: C, 84.03; H, 12.23. Found: C, 84.28; H, 12.10; mp 141-142°C, lit[24]: 142-143°C; ^1H NMR (400MHz, $CDCl_3$) δ (ppm) -<u>major isomer</u>- 0.72, 0.79, 0.82, 0.85 (4s, 12H, quaternary CH_3), 0.96 (2s, 6H, quaternary CH_3), 1.05 (d, *J* = 6.4Hz, 3H, H-30), 3.39 (dd, *J* = 10.6 and 6.7 Hz, 1H, H-29), 3.63 (dd, *J* = 10.6 and 3.2 Hz, 1H, H-29, -<u>minor isomer</u>- 0.93 (d, *J* = 6.7Hz, 3H, H-30), 3.35 (dd, *J* = 10.6 and 7.2 Hz, 1H, H-29), 3.74 (dd, *J* = 10.6 and 3.6 Hz, 1H, H-29); GC-MS of the mixture (70eV) *m/z* (rel. int.) 428 (M^+, 7%), 413(4), 369(8), 207(100), 191(61), 149(26).

Hopan-29-al 13a and **13b**: 192 mg of pyridinium dichromate (0.51 mmol) were added to 145 mg of a mixture of the two isomers **12a** and **12b** solubilised in CH_2Cl_2. After stirring a night under argon, the mixture was filtered over silica gel and the solvent removed *in vacuo* to furnish 135 mg of a mixture of the two aldehydes **13a** and **13b** (94% overall yield) which were not separable by silica gel liquid chromatography, but could be distinguished by GC (in a ratio 60:40). R_F=0.70 (hexane/ethyl acetate 9:1); Anal. calcd. for $C_{30}H_{50}O_1$: C, 84.43; H, 11.82. Found: C, 84.52; H, 11.80; mp 170-171°C, lit.[24]: 171.5-172.5°C; ^1H NMR (400MHz, $CDCl_3$) δ (ppm) 0.72-0.98 (6s, 18H, quaternary CH_3), 1.10 (d, *J* = 6.7Hz, 3H, H-30), 9.43 (d, *J* = 4.3Hz, 1H, C<u>H</u>O **13b**), 9.56 (d, *J* = 4.0Hz, 1H, C<u>H</u>O **13a**); GC-MS (70eV) *m/z* (rel. int.) -<u>first-eluting major isomer</u> **13a**- 426 (M^+, 7%), 411(3), 369(3), 205(90), 191(100), 149(22) -<u>second-eluting minor isomer</u> **13b**- 426 (M^+, 4%), 411(2), 369(3), 205(100), 191(80), 149(17).

Hopan-30-ylidene methyl acetate 14a and **14b**: 153 mg of methyl (triphenylphosphoranylidene)acetate (0.46 mmol) were mixed to 130 mg (0.31 mmol) of a mixture of the two aldehydes **13a** and **13b**. The solid was heated at 170°C under argon for 20 min. After cooling to RT and addition of CH_2Cl_2, the crude was washed with water. The organic phase was dried over $MgSO_4$ and the solvent removed under reduced pressure. TLC (CH_2Cl_2) of the crude afforded 129 mg (89% overall yield) of the two isomers **14a** and **14b** which could be separated on silica gel impregnated with 10% of silver nitrate (developer: CH_2Cl_2). **14a**: R_F=0.70 ($AgNO_3$ 10%, CH_2Cl_2); ^1H NMR (400MHz, $CDCl_3$) δ (ppm) 0.69, 0.79, 0.81, 0.85, 0.95 (5s, signal at 0.95 consists of two superimposed s, 18H, quaternary CH_3), 1.05 (d, *J* = 6.4Hz, 3H, H-29), 2.38-2.47 (m, 1H, H-22), 3.72 (s, 3H, -OC<u>H</u>$_3$), 5.70 (d, *J* = 15.5Hz, 1H, H-31), 6.73 (dd, *J* = 15.5, 9.8Hz, 1H, H-30); CG-MS (70eV), *m/z* (rel. int.) 482(M^+, 3%), 467(2), 369(10), 191(100), 149(28). **14b**: R_F=0.64 ($AgNO_3$ 10%, CH_2Cl_2); ^1H NMR (400MHz, $CDCl_3$) δ (ppm) 0.72, 0.79, 0.81, 0.84, 0.91, 0.93 (6s, 18H, quaternary CH_3), 0.94 (d, *J* = 6.6Hz, 3H, H-29), 2.28-2.38 (m, 1H, H-22), 3.73 (s, 3H, -OC<u>H</u>$_3$), 5.73 (d, *J* = 15.7Hz, 1H, H-31), 6.92 (dd, *J* = 15.7, 9.4Hz, 1H, H-30); CG-MS (70eV), *m/z* (rel. int.) 482(M^+, 2%), 369(13), 261(65),

231(8), 191(100), 149(30).

3-(30-norhopan-22-yl)-propanoic acid, methyl ester 15a and **15b**: 28 mg of PtO_2 were added to 128 mg of a mixture of compounds **14** solubilised in a mixture of hexane and diethyl ether, which was stirred under hydrogen for 8 hours. After purging with argon, the crude was filtered through celite and the solvent removed *in vacuo* affording 115 mg (90% yield) of the two isomers **15a** and **15b** which were not separable by TLC, but could be distinguished by GC, in a ratio of 50:50. R_F=0.60 (hexane/ethyl acetate 9:1 v/v); Anal. calcd. for $C_{33}H_{56}O_2$: C, 81.75; H, 11.65. Found: C, 81.53; H, 11.47; mp 126°C, lit.[24]: 125-126°C; ^{1}H NMR of the mixture (400MHz, $CDCl_3$) δ (ppm) 0.69, 0.79, 0.81, 0.85, 0.95 (6s, signal at 0.95 consists of two superimposed s, 18H, quaternary CH_3), 0.81 and 0.93 (2d, J = 6.4Hz, 3H, H-29), 2.40-2.16 (m, 3H), 3.65 and 3.66 (2s, 3H, -OC$\underline{H}_3$) ; GC-MS (70eV), *m/z* (rel. int.) -first-eluting compound- 484(M^+, 1%), 469 (1), 369(6), 263(100), 191(61), 149(14) -second-eluting compound- 484(M^+, 1%), 469 (1), 369(6), 263(100), 191(62), 149(14).

3-(17,21-epoxy-30-norhopan-22-yl)-propanoic acid, methyl ester 2a and **2b**: To a stirred solution of 100 mg (0.21 mmol) of the mixture of compounds **15** in chloroform were added 324 mg (1 mmol) of 3-chloroperoxybenzoic acid and the reaction mixture was stirred at room temperature for 120 h. The product was extracted with ether, washed with a saturated $NaHCO_3$ solution and dried over Na_2SO_4. Solvent removal, followed by preparative TLC (CH_2Cl_2) gave 26 mg (25% yield) of the mixture of the epoxides **2a** and **2b** which are not separable by TLC or by GC. R_F=0.28 (CH_2Cl_2); ^{1}H NMR of the mixture (400MHz, $CDCl_3$) δ (ppm) 0.80, 0.83, 0.85, 1.03, 1.05 (5s, 15H, quaternary CH_3), 0.95 (d, J = 7.0Hz, 3H, H-29), 2.28-2.32 (m, 1H), 2.39-2.45 (m, 2H), 3.66 (s, 3H, -OC$\underline{H}_3$) -minor isomer- 1.08 (d, J = 6.9Hz, 3H, H-29), 3.67 (s, 3H, -OC$\underline{H}_3$; GC-MS (70eV), *m/z* (rel. int.) 498(M^+, 13%), 383 (24), 275(15), 231(15), 205(25), 191(100), 149(33).

3-[30-norhopa-15,17(21)-dien-22-yl]-propanoic acid, methyl ester 16a and **16b**: To 15 mg of iodine (0.059 mmol) and 15 mg of triphenylphosphine (0.057 mmol) in 5 ml of CH_2Cl_2 were added 10 mg (0.052 mmol) of the mixture of epoxides **11** dissolved in the same solvent. After stirring for 2h under argon, the reaction mixture was worked up by washing with a saturated $NaHCO_3$ solution and dried over Na_2SO_4. The crude product was subjected to TLC (CH_2Cl_2) to give 6 mg of a mixture of 2 isomers **16a** and **16b** separable by GC. R_F=0.80 (CH_2Cl_2); ^{1}H NMR of the mixture (400MHz, $CDCl_3$) δ (ppm) 0.79 to 1.15 (methyl area), 2.15-2.20 (m, 3H), 3.63 and 3.64 (s, 3H, -OC$\underline{H}_3$), 5.63 (d, J = 10.0Hz, 1H, H-15), 6.12 (d, J = 10.0Hz, 1H, H-16); GC-MS (70eV), *m/z* (rel. int.) -first-eluting compound- 480(M^+, 5%), 465(56), 433(16), 391(4), 259(100), 227(61) -second-eluting compound- 480(M^+, 6%), 465(39), 433(14), 391(4), 259(100), 227(53).

3-[30-norhop-17(21)-en-22-yl]-propanoic acid, methyl ester 1a and **1b**: About 5 mg of Pd/C 10% was added to 13 mg (0.027 mmol) of the mixture **16** dissolved in ethyl acetate. The reaction was stirred for 24 h under hydrogen atmosphere. After purging with argon, the crude was isolated by filtration to afford 10 mg of different isomers (77% yield). The crude was chromatographed on silver nitrate impregnated silica gel plate with CH_2Cl_2 to afford the two major products **1a** and **1b**. First isomer: R_F=0.6 ($AgNO_3$ 10%, CH_2Cl_2); GC-MS (70eV), *m/z* (rel. int.) 482(M^+, 20%), 467(14), 451(4), 367(60), 231(100), 191(51). Second isomer: R_F=0.46 ($AgNO_3$ 10%, CH_2Cl_2) ^{1}H NMR (400MHz, $CDCl_3$) δ (ppm) 0.79, 0.83, 0.85, 0.86, 0.93, 1.05 (6s, 18H, quaternary CH_3), 1.01 (d, J =6.9Hz, 3H, H-29), 2.06-2.26 (m, 4H, H-16 and H-20), 3.65 (s, 3H, OC$\underline{H}_3$) ; MS (GC, 70eV), *m/z* (rel. int.) 482(M^+, 25%), 467(14), 451(4), 367(100), 231(89), 191(55).

3-[30-nor-17,21-secohopan-17,21-dion-22-yl]-propanoic acid, methyl ester 6a and **6b**: A solution of ruthenium tetroxide in carbone tetrachloride was added dropwise to 4 mg of the alkenes **1** and the reaction was stirred for 2h. After filtering the crude through silica gel, solvent removal followed by TLC (CH_2Cl_2) gave 2.5 mg of the two isomers **6a** and **6b** not separable by TLC. The GC analysis shows the presence of the two isomers in a ratio of about 50:50. R_F=0.20 (CH_2Cl_2); ^{1}H NMR of the mixture (400MHz, $CDCl_3$) δ (ppm) 0.80, 0.86, 1.01, 1.05 (6s, signals at 1.01 and 1.05 consist of two superimposed s, 18H, quaternary CH_3), 1.07 and 1.08 (2d, J = 7Hz, 3H, H-29), 2.38-2.42 (m, 2H), 2.53-2.60 (m, 2H), 3.66 and 3.67 (2s, 3H, OC$\underline{H}_3$) ; MS (GC, 70eV), *m/z* (rel. int.) -first-eluting compound- 514(M^+, 3%), 496(5), 483(4), 399(11), 344(58), 231(8), 191(100) -second-eluting compound- 514(M^+, 3%), 496(7), 483(5), 399(12), 344(53), 231(9), 191(100).

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