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Triterpenoid Chlorin Esters: Water Column Transformation Products of Chlorophyll *a*

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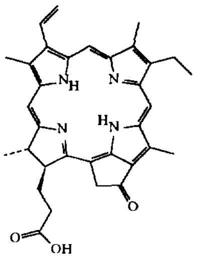
Abstract: The (22*R*)-30a,30b-dihomohopan-30b-yl and tetrahymanyl esters of pyrophaeophorbide *a*, chlorophyll *a* transformation products resulting from zooplankton herbivory, occur in an extract of surface sediment from Lake Valencia (Venezuela). The former was identified by HPLC-PDA-MS comparison with a standard and the latter after reduction of the extract and GC-MS analysis of the released alcohols.

Steryl chlorin esters (SCEs) occur almost ubiquitously in present day marine and lacustrine bottom sediments[1,2]. The first identification[3] (24-ethyl-4α-methyl-5α-cholestan-3β-ol ester of pyrophaeophorbide *a*) of a component of this type came from ^{1}H NMR spectroscopy of the chlorin nucleus of the ester isolated from a sediment of Miocene age and GC-MS analysis of the alcohol released by hydrolysis.

Subsequent work in this laboratory has included assignment of SCEs by co-injection of standards with natural mixtures using reversed phase negative ion HPLC-PDA-MS[4]. The mass spectra all show the same fragmentation pattern, *i.e.* loss of the sterol as the sterene from the molecular ion to give an ion at *m/z* 534 corresponding to the pyrophaeophorbide *a* (**1**) nucleus, a known transformation product of chlorophyll *a* (**2**), and a further peak at *m/z* 516 due to loss of water from *m/z* 534. SCEs occur as complex mixtures of sterols with pyrophaeophorbide *a* ($\lambda_{max.}$ 412, 508, 538, 610, 666 nm in acetone/methanol/water 90/5/5) as essentially the sole esterified chlorin acid. The distributions of the esterified sterols in the mixtures have been shown to represent more accurately the original water column sterol distribution at the time of deposition than do the free sedimentary sterols, thereby demonstrating the importance of the components as indicators of past phytoplankton community structures[2,5]. Recent work has shown SCE formation to occur during herbivory of a marine diatom by a copepod[6]. The SCEs in the faecal pellets consisted of a mixture of the diatom sterols, in approximately the same relative abundance as in the substrate, and the major zooplankton sterol (cholesterol, **3**) all esterified to pyrophaeophorbide *a*.



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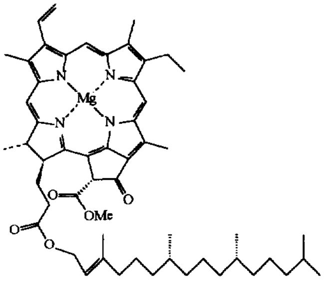

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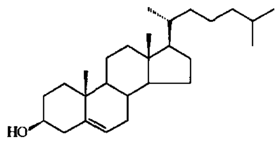

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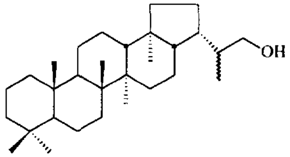
Results and Discussion

Analysis of Lake Valencia Sample: HPLC-PDA-MS analysis of a chlorin ester fraction from a sedimentary solvent extract from Lake Valencia, a highly eutrophic lake in Venezuela, revealed the presence of components less polar than pyrophaeophytin *a* (7) with negative ion mass spectra and on-line electronic spectra characteristic of SCEs but with molecular ions at *m/z* 944 and *m/z* 972, corresponding to, *inter alia*, C_{30} and C_{32} saturated pentacyclic alcohols (**4** and **5**) esterified to pyrophaeophorbide *a*. The high abundance of cyanobacteria within the lake supported the argument for the formation of hopanoid chlorin esters since cyanobacteria contain solely chlorophyll *a* as the light harvesting tetrapyrrole and, like several other types of aerobic bacteria, biosynthesise hopanoids such as bacteriohopanetetrol (**6**) as cell membrane rigidifiers[7].



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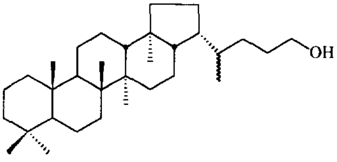

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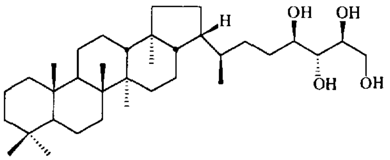

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Synthesis of hopanoid chlorin esters (Figure 1): A mixture of C-22 epimeric hopan-29-ols (**4**) was prepared by hydroboration of hop-22(29)-ene (**8**). Oxidation of the alcohols (**4**) using pyridinium dichromate with a molecular sieve 'catalyst'[8] gave a quantitative yield of epimeric hopan-29-als (**9**) which were converted into the epimeric *trans*-methyl-30a,30b-dihomohop-30-en-30b-oates (**10**) through a Wittig type reaction. Yields of this step were dependent on the degree of mixing of reagents prior to heating at 170°C *without* solvent[9]. Subsequent hydrogenation and ester reduction gave a 1:1 mixture of (22*R*)- and (22*S*)- 30a,30b-dihomohopan-30b-ol (**5**). Following hydrolysis of pyrophaeophytin *a* (**7**), pyrophaeophorbide *a* (**3**) was esterified to **4** and **5** using dicyclohexylcarbodiimide as dehydrating agent to form C-22 epimeric C_{30} and C_{32} hopanoid chlorin esters (**11** and **12**) in a manner analogous to that employed for the SCEs[4].

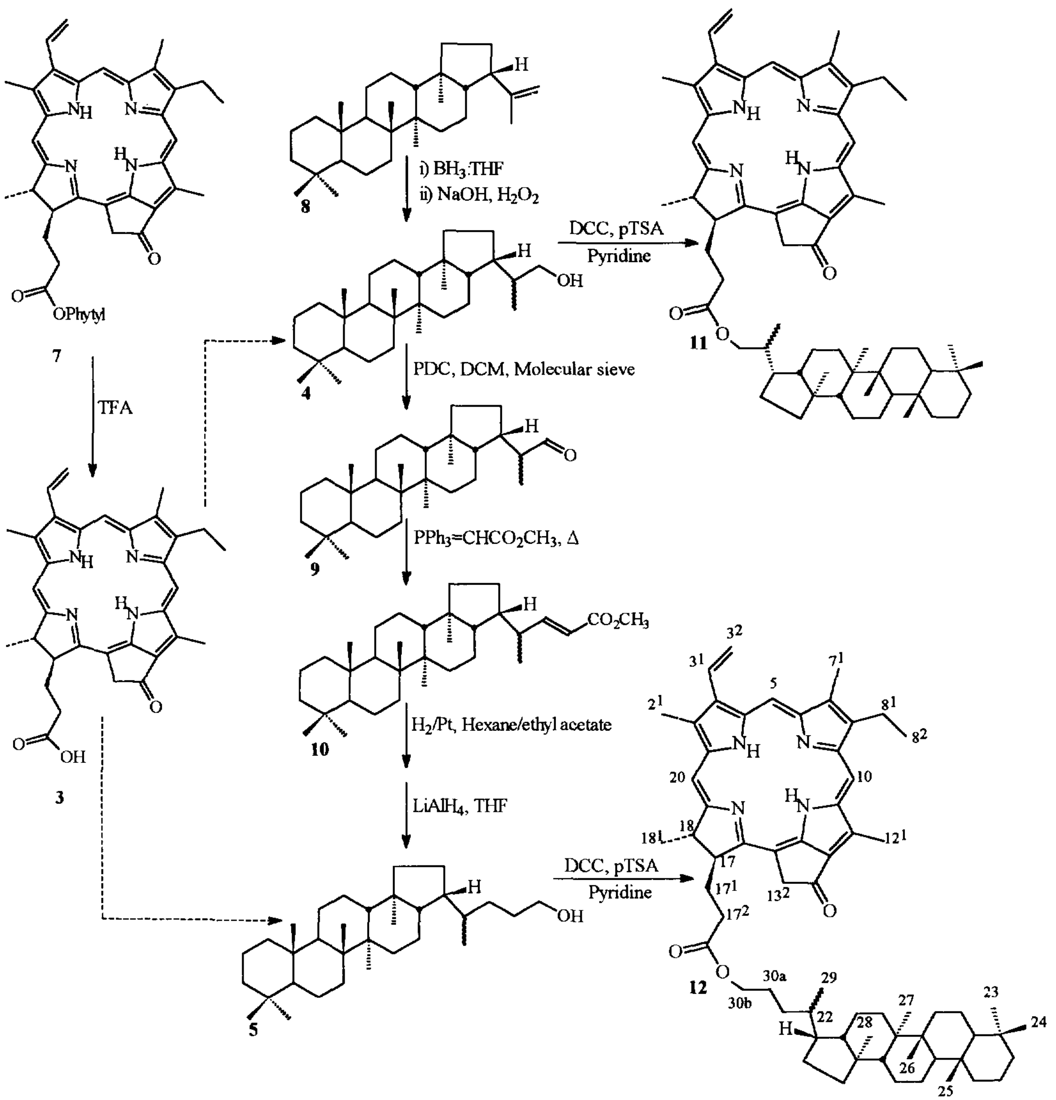


Figure 1: Synthetic scheme for preparation of hopanoid chlorin esters

Separation of C-22 epimers: The (22*R*)- and (22*S*)- hopan-29-yl esters (**11**) were only partially resolved under our standard reversed phase HPLC conditions[10]; the (22*R*)- and (22*S*)- 30a,30b-dihomohopan-30b-yl esters (**12**), however, separated by an unexpected *c.* 12 min under the same conditions (Figure 2). These were separately collected by HPLC, hydrolysed and the acetates of the alcohols made. Subsequent ^{1}H NMR using phase sensitive ^{1}H-^{1}H COSY experiments revealed the first eluting ester to be the (22*S*)-

diastereoisomer and the later eluting to be the (22*R*)- diastereoisomer (by comparison with the results of Rohmer and Ourisson[11]).

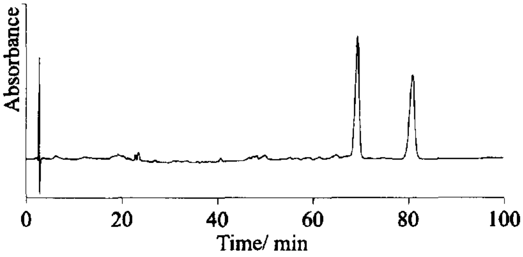


Figure 2. Analytical scale HPLC (400 nm) of mixture of (22*R*)- and (22*S*)- 30a,30b-dihomohopan-30b-yl esters of pyrophaeophorbide *a* (**12**)

Characterisation of m/z 972 hopanyl pyrophaeophorbide a esters (Table 1): The ^{1}H NMR spectra of both the (22*S*)- and the (22*R*)- isomers showed significantly different chemical shifts for the methyl groups of the hopanoid moiety when compared with the spectra of the corresponding acetates (Figure 3). In the case of the (22*S*)-30a,30b-dihomohopan-30b-ol ester of pyrophaeophorbide *a* all of the methyl groups were shielded in both deuterochloroform and deuterobenzene relative to the acetate, with particularly large increments observed for 26-H_3 (0.21 ppm in $CDCl_3$ and 0.39 ppm in C_6D_6) and 27-H_3. In the case of the (22*R*)- isomer the methyl groups were again shielded although the effects was not as large as those for the (22*S*)- isomer. Furthermore, the major effect was now observed for 28-H_3 (0.16 ppm in $CDCl_3$ and 0.21 ppm in C_6D_6) and 29-H_3.

These observations suggest that the entire hopanoid residue sits above the tetrapyrrole in the shielding region of the aromatic ring system in the (22*S*)- isomer, whilst in the (22*R*)- isomer it is only rings D and E of the hopanoid residue. Evidence for differing twisting about the ester linkage came from the signals for 30b-H_2 of the hopanoid moiety. In the (22*R*)- isomer they have identical chemical shifts (3.93 ppm) and appear as a triplet with a coupling constant of 6.5 Hz, whilst in the (22*S*)-diastereoisomer they are observed separately at *c.* 3.84 and 3.94 ppm as doublets of triplets ($^2J=10.6$ Hz, $^3J=6.5$ Hz) showing strong second order features. Attempts to prove the above hypotheses through nOe difference studies by separately irradiating the *meso*-hydrogens and β-methyls on the tetrapyrrole and methyls on the hopanoid were inconclusive. Clear enhancements were observed for signals due to protons within 3-4 Å of the irradiated protons both around the tetrapyrrole periphery, allowing confirmation of the chlorin, and between the hopanoid methyl groups. No connectivities were observed between the tetrapyrrole and the hopanoid in the case of the (22*R*)- isomer. In the case of the (22*S*)- isomer there was, however, a connectivity between one of the hopanoid hydrogens and 5-H and 7^1-H_3 of the tetrapyrrole. Unfortunately, the identity of this hydrogen could not be established from the phase sensitive ^{1}H-^{1}H COSY spectrum. A further connectivity was apparent through a negative nOe between 26-H$_3$ of the hopanoid and one of the pyrrole N-H's. It is tempting to suggest, therefore, that the hopanoid moiety sits above the tetrapyrrole in the (22*S*)- isomer, in closer proximity than in the (22*R*)- isomer.

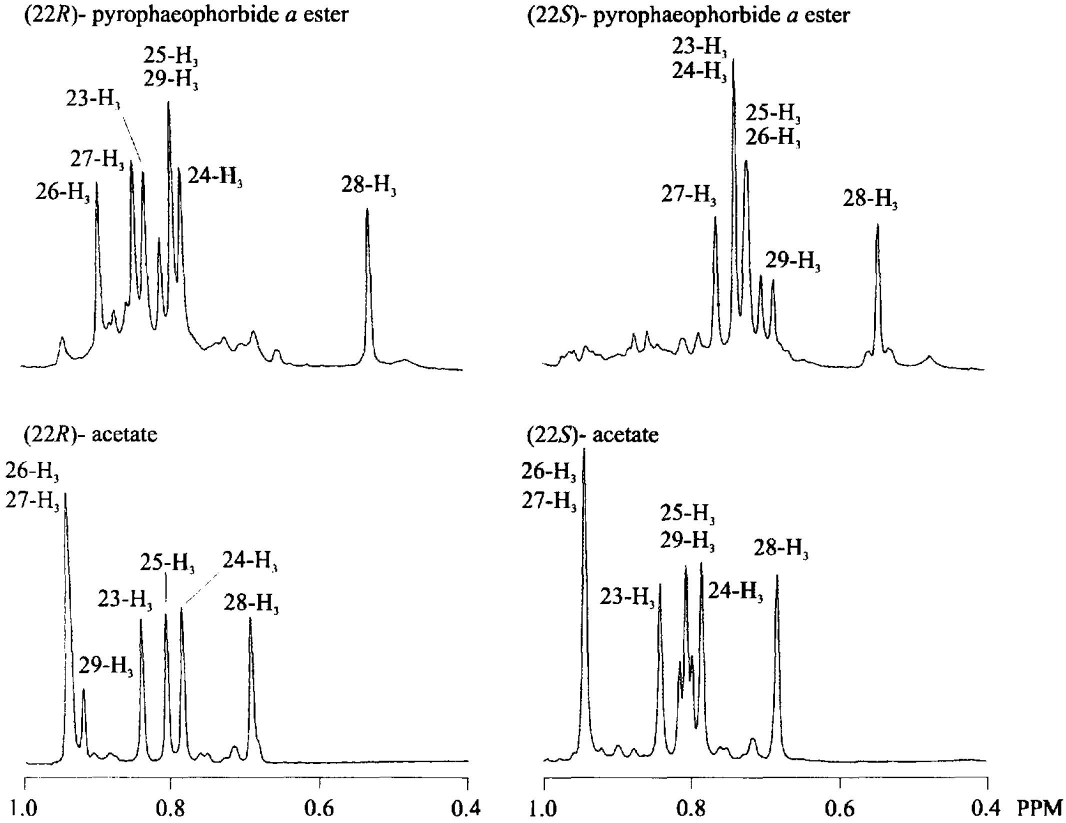


Figure 3. Partial ^{1}H NMR spectra (400 MHz) of 30a,30b-dihomohopan-30b-yl esters showing the methyl group resonances of the hopanoid (CDCl$_3$)

Interestingly, the nOe experiments also revealed the relative chemical shifts of the tetrapyrrole *meso*-hydrogens to be different in each solvent for both diastereoisomers such that in deuterochloroform; H-10 is more deshielded than H-5 whereas in deuterobenzene the reverse is true[5]. The same effect occurs for 2^1H$_3$ and 7^1-H$_3$ (Table 1).

Table 1. ^{1}H NMR chemical shift data for the (22*S*)- and (22*R*)-30a,30b-dihomohopan-30b-yl esters of pyrophaeophorbide *a* and acetates

Position	(22*S*)-ester ($CDCl_3$)	(22*R*)-ester ($CDCl_3$)	(22*S*)-ester (C_6D_6)	(22*R*)-ester (C_6D_6)	(22*S*)-acetate ($CDCl_3$)	(22*R*)-acetate ($CDCl_3$)	(22*S*)-acetate (C_6D_6)	(22*R*)-acetate (C_6D_6)
2^1-H_3	3.41(s)	3.41(s)	3.15(s)	3.17(s)				
3^1-H	8.02(dd)	7.95(dd)	7.95(dd)	7.95(dd)				
$3^{2'}$-H_2 *(trans)*	6.29(dd)	6.29(dd)	6.20(dd)	6.20(dd)				
$3^{2''}$-H_2 *(cis)*	6.18(dd)	6.18(dd)	5.99(dd)	5.99(dd)				
5-H	9.39(s)	9.41(s)	9.64(s)	9.66(s)				
7^1-H_3	3.24(s)	3.25(s)	3.17(s)	3.16(s)				
8^1-H_2	3.69(q)	3.71(q)	3.61(q)	3.63(q)				
8^2-H_3	1.70(t)	1.70(t)	1.70(t)	1.70(t)				
10-H	9.51(s)	9.52(s)	9.60(s)	9.62(s)				
12^1-H_3	3.68(s)	3.68(s)	3.57(s)	3.58(s)				
$13^{2'}$-H_2	5.28(d)	5.27(d)	5.25(d)	5.27(d)				
$13^{2''}$-H_2	5.11(d)	5.11(d)	4.94(d)	4.95(d)				
17-H	4.31(m)	4.30(m)	4.05(m)	4.01(m)				
17^1-H_2 and	2.72(m),	2.72(m),	2.65(m),	2.63(m),				
17^2-H_2	2.53(m),	2.53(m),	2.48(m),	2.48(m),				
	2.31(m)	2.31(m)	2.32(m)	2.28(m)				
18-H	4.49(dq)	4.49(dq)	4.30(dq)	4.24(dq)				
18^1-H_3	1.81(d)	1.81(d)	1.64(d)	1.62(d)				
20-H	8.55(s)	8.56(s)	8.49(s)	8.48(s)				
23-H_3	0.74(s)	0.83(s)	0.79(s)	0.92(s)	0.83(s)	0.84(s)	0.97(s)	0.97(s)
24-H_3	0.74(s)	0.78(s)	0.85(s)	0.88(s)	0.78(s)	0.78(s)	0.91(s)	0.92(s)
25-H_3	0.72(s)	0.80(s)	0.78(s)	0.90(s)	0.80(s)	0.81(s)	0.92(s)	0.92(s)
26-H_3	0.72(s)	0.90(s)	0.64(s)	0.97(s)	0.93(s)	0.93(s)	1.03(s)	1.04(s)
27-H_3	0.76(s)	0.85(s)	0.73(s)	0.96(s)	0.93(s)	0.93(s)	1.04(s)	1.02(s)
28-H_3	0.55(s)	0.53(s)	0.57(s)	0.55(s)	0.67(s)	0.69(s)	0.77(s)	0.76(s)
29-H_3	0.69(d)	0.80(d)	0.75(d)	0.85(d)	0.80(d)	0.92(d)	0.85(d)	0.98(d)

Assignment of hopanyl pyrophaeophorbide a esters in Lake Valencia sediment: Coinjection of the mixture of (22*R*)- and (22*S*)- hopan-29-yl esters with a chlorin ester fraction from Lake Valencia surface sediment using HPLC-PDA-MS showed that neither diastereoisomer was present, the standard eluting *c.* 5 min after the target *m/z* 944 component. Coinjection of the (22*R*)-30a,30b-dihomohopan-30b-yl ester produced enhancement (Figure 4) of the peak at 81.73 min (400 nm) and an increase in the relative intensity of the parent ion (*m/z* 972) in the mass chromatogram. The on-line electronic spectrum remained unchanged.

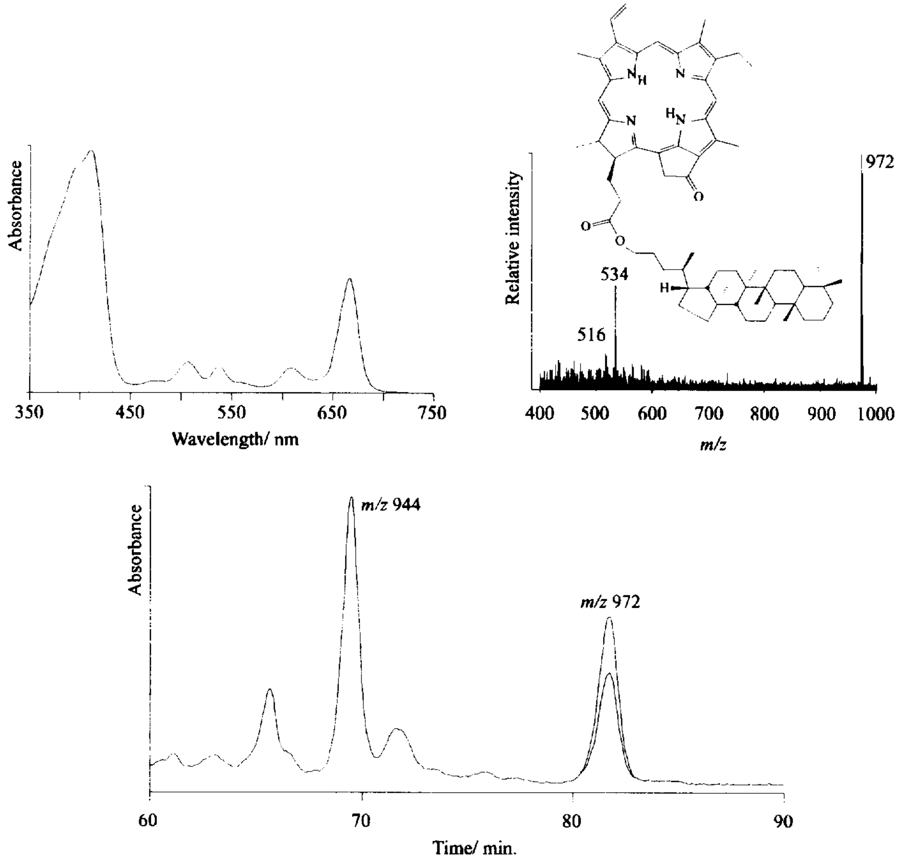
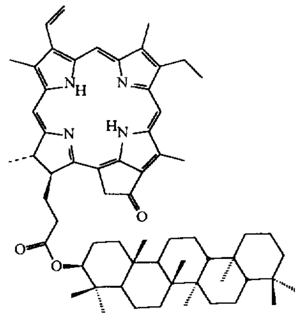


Figure 4. HPLC chromatogram (400 nm), electronic and mass spectra of (22*R*)-30a,30b-dihomohopan-30b-yl ester of pyrophaeophorbide *a* and coinjection with Lake Valencia sediment fraction.

Other triterpenoid chlorin ester: In order to identify the *m/z* 944 component the fraction was reduced with $LiAlH_4$ in order to release the alcohols which were analysed as their TMSi ethers by GC-MS. The mixture contained not only the expected (22*R*)-30a,30b-dihomohopan-30b-ol but also tetrahymanol as confirmed by coinjection with a standard (insufficient standard was available for synthesis of the pyrophaeophorbide *a* ester). Since this was the only alcohol in the reduced fraction with a molecular weight corresponding to the esterifying alcohol in the *m/z* 944 ester, it was concluded that the major triterpenoid chlorin ester in the extract was the tetrahymanyl ester of pyrophaeophorbide *a* (**13**). This assignment is reflected in the HPLC retention behaviour of the ester compared with that of the synthesised hopanoid chlorin esters (the



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program is isocratic after 40 min 90:5:5, acetone: methanol: water) and of the free alcohols under GC analysis, where the hopanoids elute with the (22*S*)- isomer eluting before the (22*R*)- [†] and the isomer separation increases with side chain length. Tetrahymanol eluted before the (22*R*)- and (22*S*)-hopan-29-ol isomers (GC) as did the *m/z* 944 component relative to the hopan-29-yl esters of pyrophaeophorbide *a* (HPLC).

Origins and distributions of triterpenoid chlorin esters: The source of the C_{32} alcohol is presumably bacteriohopanetetrol (**6**) from the abundant cyanobacterial population of the lake; no organisms have been found to contain 30a,30b-dihomohopan-30b-ol[12]. Since SCE formation has been demonstrated to occur within 48 h[6] during zooplankton grazing, it appears that degradation of the polyfunctionalised side chains of bacteriohopanetetrol derivatives occurs in the water column. This transformation may also be induced by herbivory. The pyrophaeophorbide *a* is derived from transformation of chlorophyll *a*, but it is unclear whether the esterification occurs specifically with ingested cyanobacterial chlorophyll or with ingested chlorophyll present in the zooplankton gut from a mixed phytoplanktonic diet.

Tetrahymanol occurs almost ubiquitously in marine and lacustrine sediments[13,14,15] and is of ciliate origin; ciliates only biosynthesise tetrahymanol when there is, however, no available dietary sterol source[16]. It is not clear whether the tetrahymanyl ester is formed by the mixed grazing of zooplankton on phytoplankton (containing chlorophyll *a*) and ciliates (containing tetrahymanol) or whether ciliate grazing of cyanobacteria might initiate chlorin ester formation. If the latter were the case then such grazing might also be a means of formation of both 30a,30b-dihomohopan-30b-ol and its pyrophaeophorbide *a* ester.

Conclusions: The (22*R*)-30a,30b-dihomohopan-30b-yl ester of pyrophaeophorbide *a* occurs in the surface sediment of lake Valencia. The corresponding tetrahymanyl ester was identified by reduction of the extract and characterisation of the released alcohol by GC-MS. The hopanyl ester is presumably of cyanobacterial origin although whether the chlorin nucleus is derived specifically from a cyanobacterial diet or a mixed phytoplanktonic diet is unclear. The formation of this ester, containing a bacteriohopanetetrol derivative, indicates a water column transformation which is probably zooplankton mediated. The tetrahymanyl ester presumably arises through mixed grazing of ciliates and phytoplankton.

EXPERIMENTAL

High Performance Liquid Chromatography-Photodiode Array-Mass Spectrometry: Reversed phase HPLC (Waters MS600 Silk Quaternary) used two Waters Nova-Pak C_{18} radial compression cartridges (100 mm x 5 mm i.d. each in series, 1 ml min^{-1}) with a C_{18} pre-column (10 mm x 5 mm i.d.). The use of an on-line Waters 991 PDA detector provided electronic spectra of individual components. The program used gradient elution with 90% B, 10% C isocratic (5 min), then to 70% A, 15% B, 15% C after 15 min, then to 90%

[†] For hopan-29-ol, the (22*S*)-diastereoisomer elutes after the (22*R*)-because of the priority rules used in assigning stereochemistry to chiral centres; (22*S*)-hopan-29-ol has the 'natural' stereochemistry at C-22 seen in bacteriohopanetetrol and (22*R*)-30a,30b-dihomohopan-30b-ol.

A, 5% B, 5% C after 40 min and isocratic thereafter, where A= acetone, B= methanol and C= water. The system was linked to a Finnigan MAT TSQ 700 quadruple mass spectrometer, linked via a Finnigan MAT TSP-2 thermospray interface[1].

Combined Gas Chromatography-Mass Spectrometry: GC-MS was performed with a Varian 3400 gas chromatograph with a septum equipped, temperature programmable injector (SPI). The injector was raised from 30°C to 300°C at 200°C min^{-1} and kept at 300°C for the length of the temperature program [40-200°C (10°C min^{-1}) - 300°C (3°C min^{-1}, hold 30 min]. A CP Sil-5CB column (50 m) was used and the carrier gas was hydrogen. The GC was coupled to the TSQ 700 with a source temperature of 170°C, transfer line temperature of 300°C, electron energy of 70 eV, electron current of 400 μA and scanning Q3 from *m/z* 50-650 per second. Data acquisition was performed using an ICIS data system.

^1H NMR: ^1H NMR spectra were recorded on Jeol GX270 and GX400 series instruments at ambient temperature. The spectra were recorded in $CDCl_3$ unless otherwise stated and referenced to the solvent peak (or TMS for strong samples). For chlorin esters, ^1H NMR spectra were acquired with a pulse width of *c.* 60° and a typical pulse delay of 0.5 s. and processed using a Gaussian window function.

Extraction of Lake Valencia Surface Sediment:[10] The frozen sample was allowed to thaw and excess water was removed by centrifugation. The samples were extracted ultrasonically with doubly distilled acetone. Following centrifugation, the supernatant was decanted and the extraction procedure repeated until the supernatant was colourless (typically 6 times). The combined extracts were filtered (Whatman no. 1). Fractions concentrated in chlorin esters were obtained by two preparative steps: (i)Gel permeation HPLC (Polymer Laboratories PL-Gel 50 Å, 600 x 7.5 mm i.d.; methanol/ dichloromethane 51/49 v/v isocratic at a flow rate of 1.5 $ml min^{-1}$) with UV/Vis detection at 660 nm, followed by (ii) reversed phase HPLC of the fraction from (i) collected over the retention time range 43-50 min (Waters Radial Compression Cartridge, 100 x 25 mm i.d.; acetone/ methanol 90/10 v/v isocratic at a flow rate of 5.0 $ml min^{-1}$), by using UV/Vis detection at 660 nm and collecting the fraction eluting from 11 to 30 min.

(22R)-, (22S)-Hopan-29-ol (4):[17] Hop-22(29)-ene (**8**, 1.17 g, 2.84 mmol) was dissolved in a minimum volume of tetrahydrofuran (distilled from sodium wire under nitrogen) at 0°C under a nitrogen atmosphere. Borane:THF complex (2.6 ml, 2.6 mmol) was added and the reaction stirred under nitrogen at 0°C for 3 h. Sodium hydroxide (1 M, 0.85 ml) was added carefully until gas evolution ceased, followed by dropwise addition of a 20% excess of 30% hydrogen peroxide. The mixture was poured into water (200 ml) and extracted with diethyl ether (3 x 200 ml). The combined ether layers were washed with saturated sodium bicarbonate solution and dried over anhydrous magnesium sulphate. The ether was removed under reduced pressure to give hopan-29-ol (**4**) as a white solid (1.19 g, 98%) δ_H(400MHz) 3.76-3.32 (4 x dd, J 10.5, 3.4 and J 10.5, 6.4, 29-H_a and 29-H_b), 1.05 (d, J 6.6, 30-H_3), 0.95 (s, 26-H_3 and 27-H_3), 0.92 (d, J 6.6, 30-

H$_3$), 0.85 (s, 25-H$_3$), 0.81 (s, 23-H$_3$), 0.79 (s, 24-H$_3$), 0.72 (s, 28-H$_3$), 0.71 (s, 28-H$_3$). Found for shorter retention time diastereoisomer *m/z* 500 (M$^+$(TMS), 4%), 485 (3), 410 (6), 369 (8), 279 (45), 207 (21), 191 (83), 189 (100) and 95 (41) and for longer retention time diastereoisomer *m/z* 500 (M$^+$(TMS), 4%), 485 (3), 410 (8), 369 (6), 279 (62), 207 (6), 191 (77), 189 (100) and 95 (39).

(22R)-, (22S)-Hopan-29-al (9):[8] Molecular sieve (Sigma, 3Å) was crushed using a pestle and mortar, stirred for 8 h. under high vacuum and stored in an oven at 140°C. Pyridinium dichromate (263 mg, 1.5 eq., 700 µmol) and molecular sieve (470 mg) were placed in the reaction vessel under a nitrogen atmosphere. Sufficient dry dichloromethane was added to make a suspension of the pyridinium dichromate and molecular sieve (*c.* 2 ml). To this was added hopan-29-ol (**4**, 200 mg, 470 µmol) in dichloromethane (20 ml) and the mixture stirred at room temperature for 1h after which the reaction was shown to be complete by TLC. The mixture was diluted with ether and filtered through 10% calcium sulphate in silica. The solvent was removed under reduced pressure to give the product as a white solid (199 mg, 100%). δ$_H$(400MHz) 9.56 (d, J 4.0, 29-H (22*R*)), 9.43 (d, J 5.4, 29-H (22*S*)), 1.10 (d, J 6.7, 30-H$_3$), 0.96 (s, 26-H$_3$), 0.94 (s, 27-H$_3$), 0.92 (s, 27-H$_3$), 0.85 (s, 25-H$_3$), 0.84 (s, 25-H$_3$), 0.82 (s, 23-H$_3$), 0.81 (s, 23-H$_3$), 0.79 (s, 24-H$_3$), 0.79 (s, 24-H$_3$), 0.73 (d, J 0.6, 28-H$_3$), 0.71 (d, J 0.6, 28-H$_3$). Found *m/z* 426 (M$^+$, 9%), 411 (5), 369 (7), 205 (100), 191 (97), 147 (40), and 95 (42).

(22R)-, (22S)-Methyl-30a,30b-dihomohop-30-en-30b-oate (10):[18] Hopan-29-al (**9**, 200 mg, 469 µmol) and methyl triphenylphosphoranylidene acetate (234 mg, 700 µmol) were mixed thoroughly with a pestle and mortar until a very fine white powder was obtained. The solid mixture was transferred to a round bottomed flask and heated to 170°C under an atmosphere of nitrogen. After 30 min the black oily product was allowed to cool and extracted with dichloromethane. The solution was washed with saturated sodium bicarbonate solution and dried over anhydrous magnesium sulphate. Solvent was removed under reduced pressure to give a crude mixture which was purified using silica column chromatography eluting with hexane/ dichloromethane (4:1) to give a white product (220 mg, 97%). δ$_H$(400MHz) 6.92 (dd, J 15.6, 9.3, H-30), 6.73 (dd, J 15.6, 9.9, H-30), 5.72 (d, J 15.6, H-30a), 5.69 (d, J 15.8, H-30a), 1.05 (d, J 6.6, 29-H$_3$), 0.94 (s, 27-H$_3$ and 26-H$_3$), 0.93 (d, J 6.6, 29-H$_3$), 0.92 and 0.90 (s, 27-H$_3$ and 26-H$_3$), 0.84 (s, 25-H$_3$), 0.835 (s, 25-H$_3$), 0.805 (s, 23-H$_3$), 0.80 (s, 23-H$_3$), 0.785 (s, 24-H$_3$), 0.78 (s, 24-H$_3$), 0.71 (s, 28-H$_3$), 0.69 (s, 28-H$_3$). Found for shorter retention time diastereoisomer *m/z* 482 (M$^+$, 7%), 467 (8), 369 (23), 261 (71), 231 (9), 191 (100), and 95 (53) and for longer retention time diastereoisomer *m/z* 482 (M$^+$, 4%), 467 (4), 369 (33), 261 (84), 231 (10), 191 (100) and 95 (58).

(22R)-, (22S)-Methyl-30a,30b-dihomohopan-30b-oate: Methyl-30a,30b-dihomohop-30-en-30b-oate (200 mg, 415 µmol) was dissolved in hexane/ ethyl acetate (2:1). Platinum oxide (100 mg) was added and the reaction stirred at room temperature under a atmosphere of hydrogen for 16 h. The product was filtered through silica and the solvent removed under reduced pressure to give a white product (**10**, 200 mg, 100%). δ_H(400MHz) 3.66 (s, -OC$\underline{H}_3$), 3.65 (s, -OC$\underline{H}_3$), 0.95 (s, 27-H$_3$ and 26-H$_3$), 0.93 (d, J 6.4, 29-H$_3$), 0.85 (s, 25-H$_3$), 0.81 (d, J 6.1, 29-H$_3$), 0.81 (s, 23-H$_3$), 0.79 (s, 24-H$_3$), 0.70 (s, 28-H$_3$), 0.69 (s, 28-H$_3$). Found for shorter retention time diastereoisomer *m/z* 484 (M^+, 3%), 469 (4), 369 (12), 263 (100), 231 (6), 191 (53) and 95 (28) and for longer retention time diastereoisomer *m/z* 484 (M^+, 3%),469 (4), 369 (13), 263 (100), 231 (7), 191 (56) and 95 (28).

(22R)-, (22S)-30a,30b-dihomohopan-30b-ol (5): Lithium aluminium hydride (25 mg, 4 eq., 640 µmol) was stirred in dry tetrahydrofuran under a nitrogen atmosphere to make a slurry. Methyl-30a,30b-dihomohopan-30b-oate (79 mg, 160 µmol) was dissolved in dry tetrahydrofuran and added to the slurry. The mixture was heated under reflux for 1h. After allowing to cool, a saturated solution of Rochelle's salt (potassium sodium tartarate) was added dropwise. The mixture was extracted with ether, but the solution had to be acidified by dropwise addition of hydrochloric acid (1 M) until any solids had dissolved to facilitate separation of the phases. The solution was washed with water, the ether dried over anhydrous magnesium sulphate and the solvent removed under reduced pressure to yield a white product (74 mg, 100%). δ_H(400MHz) 0.95 (s, 27-H$_3$ and 26-H$_3$), 0.94 (d, J 6.3, 29-H$_3$), 0.85 (s, 25-H$_3$), 0.82 (d, J 6.3, 29-H$_3$), 0.81 (s, 23-H$_3$), 0.79 (24-H$_3$), 0.70 (s, 28-H$_3$), 0.69 (s, 28-H$_3$). Found for shorter retention time diastereoisomer *m/z* 528 (M^+(TMS), 2%), 513 (3), 369 (15), 307 (59), 231 (8), 217 (97), 191 (100) and 95 (55) and for longer retention time diastereoisomer *m/z* 528 (M^+ (TMS), 3%), 513 (3), 369 (17), 307 (56), 231 (8), 217 (86), 191 (100) and 95 (52).

Pyrophaeophytin a (7):[19] Impure pyrophaeophytin *a* was obtained from the British Chlorophyll Company (BCC). Impurities (mainly carotenoids) were separated by chromatography on sucrose (*c.* 30 x 7 cm) and eluting with 0.2-0.5% n-propanol in petroleum spirit (40-60°C). Impure BCC pyrophaeophytin *a* was chromatographed in 3 x 2 g batches and the corresponding fractions combined and rechromatographed twice to afford pyrophaeophytin *a* as a dark green oily solid (70 mg (UV/Vis), *m/z* 812 (M^-, 100%)) pure with respect to other pigments (400 nm).

Pyrophaeophorbide a (3):[20] Pyrophaeophytin *a* (70 mg, 86 µmol) was dissolved in cold (0°C) aqueous trifluoroacetic acid (15 ml) through which nitrogen had been bubbled for 10 min to remove air. The resulting green solution was protected from light and stirred under a nitrogen atmosphere at 0°C for 4 h. The reaction mixture was poured into water (100 ml) and extracted with dichloromethane. The extract was washed with copious amounts of water and the solvent removed under reduced pressure to give the dark green oily product, pyrophaeophorbide *a* (50 mg (UV/Vis), 85%), *m/z* 548 ($M^{\cdot}$(Me), 100%)) which was free from other pigments (400 nm).

Triterpenoid pyrophaeophorbide a esters:[4] Pyrophaeophorbide *a* (5 mg, 9.3 μmol) was transferred to the reaction flask using dichloromethane as solvent which was removed under a stream of nitrogen. The flask was stored overnight over phosphorous pentoxide under vacuum in a desiccator in the dark. The flask was put under a nitrogen atmosphere and the appropriate alcohol (9.1 mg, 20 μmol), dicyclohexylcarbodiimide (2.8 mg, 14 μmol) and *para*-toluenesulphonic acid (*c.* 0.2 mg, 1 μmol, 1 crystal) added. The mixture was dissolved in 15 ml of dry pyridine (distilled under nitrogen from calcium hydride) and stirred at room temperature for 0.5 h in the dark. The reaction was stopped by transferring the reaction mixture to a nitrogen-filled round bottom flask and the pyridine removed under reduced pressure. The remaining reaction mixture was transferred to the round bottom flask with dry dichloromethane which was also removed under reduced pressure. Trace quantities of residual pyridine were removed by drying the product over phosphorous pentoxide under vacuum in a desiccator in the dark. The crude product was purified by semi-preparative scale HPLC (2 mlmin^{-1}; acetone/ methanol 60/40 on two Waters reverse-phase Nova Pak C_{18} radial compression cartridges of 8 mm i.d. and 10 cm length) to give dark green oily products.

To remove lipid contamination the two compounds were placed on silica columns and washed with hexane, an increasing proportion of dichloromethane and finally eluted with 1% acetone in dichloromethane.

For hopan-29-yl ester: δ_H(400MHz, CD_2Cl_2) 9.54 (s, 10-H), 9.41 (s, 5-H), 8.63 (s, 20-H), 8.05 (dd, J 12.0, 18.0, 3^1-H), 6.31 (dd, J 12.0, 1.0, 3^2-H), 6.17 (dd, J 12.0, 1.0, $3^{2'}$), 5.24 (d, J 20.0, 13^2-H), 5.07 (d, J 20.0, $13^{2'}$-H), 3.70 (q, J 7.6, 8^1-H_2), 3.64 (s, 12^1-H_3), 3.61-3.54 (m, 29'-H_2), 3.41 (s, 2^1-H_3), 2.7-2.2 (m, 17^1-H_2 and 17^2-H_2), 1.80 (d, J 7.63, 18^1-H_3), 1.79 (d, J 7.63, 18^1-H_3), 1.70 (t, J 8.24, 8^2-H_3). Found *m/z* 944 ($M^{\cdot}$, 100%) and 516 (21%). For analytical results for 30a,30b-dihomohopan-30b-ol esters see Table 1 and Figure 2 and Figure 3. On-line UV/Vis spectra for both products were identical: λ/nm 408 (100%), 506 (11), 536 (9), 608 (8) and 666 (42).

Hydrolysis of 30a,30b-dihomohopan-30b-yl esters of pyrophaeophorbide a:[21] The separated (22*S*)- and (22*R*)- esters (*c.* 2 mg) were dissolved in diethyl ether and shaken with hydrochloric acid (*c.* 20 ml, 80%v/v). The acid was washed several times with ether and the ether layers combined. The ether was washed with hydrochloric acid (*c.* 2 x 20 ml, 80% v/v) and copious amounts of water. The solvent was removed under reduced pressure. Due to the small amount of alcohol formed, the product was acetylated immediately without analysis.

(22R)- and (22S)- 30a,30b-dihomohopan-30b-yl acetate: 30a,30b-dihomohopan-30b-ol (2 mg, 4.4 μmol) was dissolved in the minimum volume of dry dichloromethane under a nitrogen atmosphere. Acetic anhydride (450 μl, 1.1 eq.), triethylamine (666 μl, 1.1 eq.) and dimethylaminopyridine (1 crystal, catalyst) were added and the reaction stirred under a nitrogen atmosphere for 15 min at room temperature. The product was extracted with diethyl ether, washed with water, dried over anhydrous magnesium sulphate and the solvent removed under reduced pressure. The crude product was purified by small scale column chromatography (Pasteur pipette) on silica eluting with 10% ethyl acetate in hexane. See Table 1 for ^{1}H NMR results. Found for the 22*S* diastereoisomer *m/z* 498 (M^+, 4%), 483 (4), 369 (13), 277 (100), 217 (12) and 191 (56). Found for 22*R* diastereoisomer *m/z* (M^+, 4%), 483 (4), 369 (16), 277 (100) and 191 (58).

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