101. Breakdown of Chlorophyll: Partial Synthesis of a Putative Intermediary Catabolite

Preliminary Communication

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Dedicated to Professor *Philippe Matile* on the occasion of his 65th birthday

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The partial synthesis of 10,22-dihydro-4,5-dioxo-4,5-secopheophorbide a (**1**) from pheophorbide a methyl ester (**2**) is described. A regioselective, photooxygenolytic reaction of (pheophorbidato a methyl ester)cadmium(II) (**3**) provides the entry to the crucial 4,5-secoporphinoid structure in form of the (10,22-dihydro-4,5-dioxo-4,5-seco-pheophorbidato a methyl ester)cadmium(II) (**4**). The hydride reduction of this 4,5-dioxo-4,5-secophytoporphyrin ester occurs selectively at the 'eastern' *meso*-position to lead (after demetallation) to 10,22-dihydro-4,5-dioxo-4,5-secopheophorbide a methyl ester (**5**). This oxobilin-carbaldehyde has the structure assigned earlier to an ester of an isolation form of the red pigment(s) from *Chlorella protothecoides*. Hydrolysis of the propanoate ester function of **5**, selectively catalyzed by pig liver esterase, then yields the title compound **1**. The red tetrapyrrole **1** may represent an intermediary chlorophyll catabolite in degreening plants.

Introduction. – In spite of its visual impressiveness and its apparent ecological relevance, breakdown of the green plant pigment chlorophyll only recently has yielded some of its secrets (see *Scheme 1*) [1]. In senescent plants, chlorophyll was shown to rapidly be degraded to colorless and nonfluorescing tetrapyrrole compounds, termed 'nonfluorescent chlorophyll catabolites' (NCCs) [2]. Contrasting all expectations [3], the structure of the first one of these to be characterized, of *Hv*-NCC-1 (**Ia**) [4] from senescent primary leaves of barley (*Hordeum vulgare*) [5], indicated as the key step an oxygenolytic cleavage at the 'northern' *meso*-position of the porphinoid chlorophyll macrocycle [6]. In the meantime, from a range of senescent plant materials, NCCs have been isolated and structurally analyzed [7–9] and they indeed have been shown to exhibit the same basic tetrapyrrole structure as first found in **Ia**.

Both structural [6] and plant physiological considerations [10] indicated the NCC's to represent 'late' and possibly final (storage) forms of chlorophyll catabolites in plants [2]. The discovery, in minute traces, of 'fluorescent' chlorophyll catabolites (FCCs), in the earlier phases of chlorophyll breakdown [11] very recently was followed by the elucidation of the constitution of *Bn*-FCC-2 (**II**), an FCC from the degreened cotyledons of rape (*Brassica napus*) [12]. Its structure confirmed its intermediary position in the course of the

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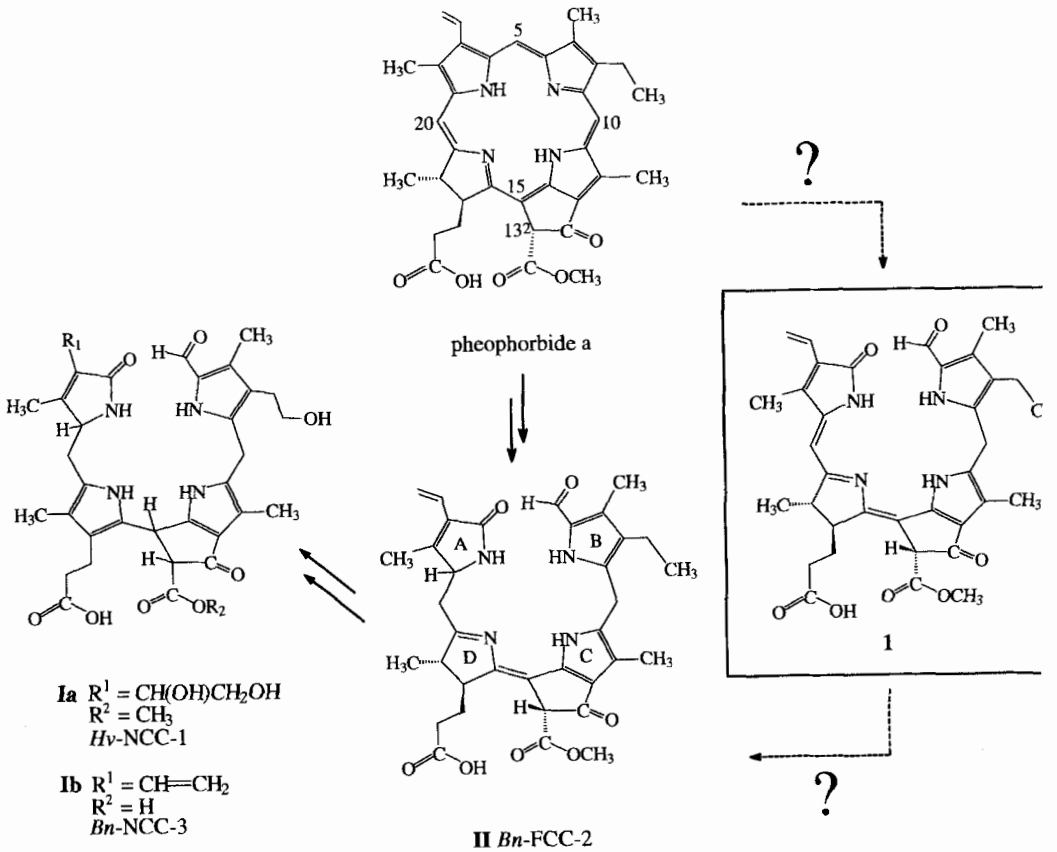
Scheme 1



breakdown of chlorophyll and helped to narrow down the possible changes occurring in its early stages: formally *Bn*-FCC-2 (**II**) can be derived from pheophorbide a by addition of one molecule of O_2 and two molecules of H_2. From **II**, further catabolism (peripheral oxidation, tautomerization, and ester hydrolysis) appears to rationally lead to the non-fluorescing catabolite *Bn*-NCC-3 (**Ib**), the presumed biosynthetic precursor to other NCCs in *Brassica napus* [7b].

Other important recent observations in the area of chlorophyll breakdown concern the secretion of red pigments by the green alga *Chlorella protothecoides*, when grown in a medium deficient of nitrogen [13]. These pigments were characterized by *Gossauer* and co-workers [14] [15] as oxobilin-carbaldehydes (formylbilinones) having the same main skeleton as that first observed with the plant catabolite *Hv*-NCC-1 (**Ia**) [4] (see *Scheme 1*), suggesting a structurally related degradation of chlorophylls in the alga and in green plants [7b] [15].

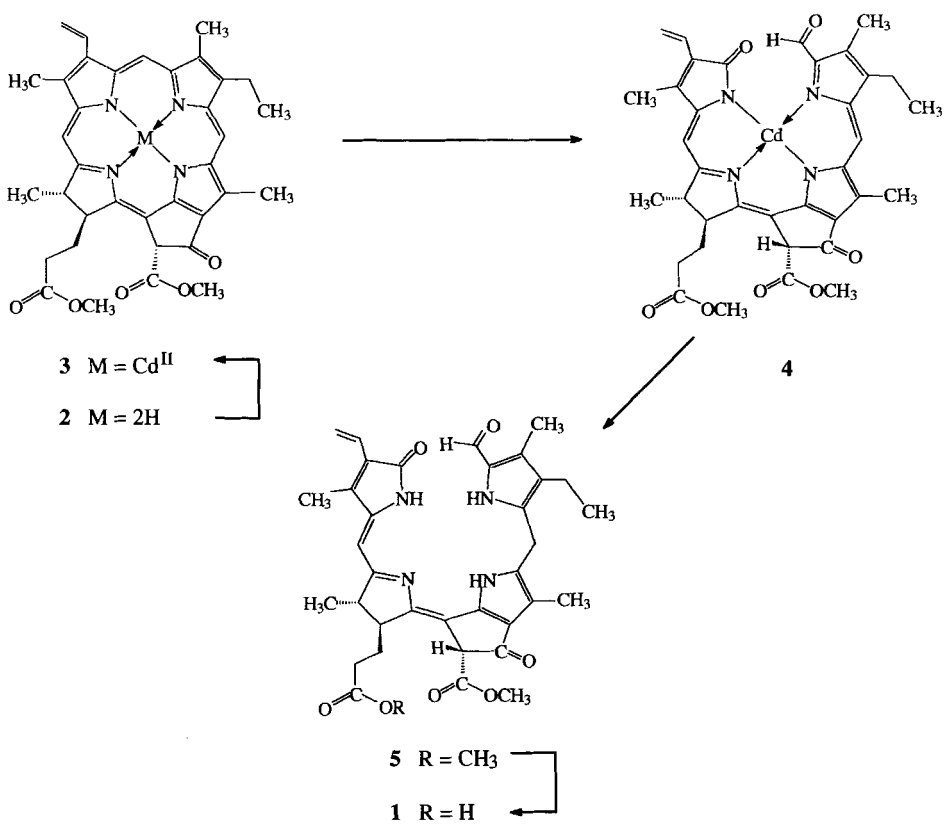
Considering all these findings, it appeared of interest to experimentally examine the possibility that red pigments related to those secreted by *Chlorella protothecoides* would represent intermediary (but still elusive) catabolites in degreening plant tissues [12]. We

report here on a first step towards this goal, on the preparation of 10,22-dihydro-4,5-dioxo-4,5-secopheophorbide a (**1**), a putative catabolic precursor of *Bn*-FCC-2 (**II**) in senescent cotyledons of rape. In addition, as the synthetic precursor of **1**, the dimethyl ester **5** was prepared. This ester was found to be identical with the ester of an isolation form of the red pigment from *Chlorella protothecoides*, characterized by *Gossauer* and co-workers [16].

Results and Discussion. – The key step in the preparation of 10,22-dihydro-4,5-dioxo-4,5-secopheophorbide a methyl ester (**5**) was the selective oxygenolytic opening of the pheophorbide macrocycle between C(4) and C(5) (*Scheme 2*). We applied the photooxygenolytic cleavage method developed by *Iturraspe* and *Gossauer* [17] for the analogous opening of the somewhat less delicate pyropheorbide a methyl ester (17^4-methyl-pyro-pheophorbidate a) and for the preparation of 13^2-de(methoxycarbonyl)-10,22-dihydro-4,5-dioxo-4,5-secopheorbide a (17^4-methyl-13^2-de(methoxycarbonyl)-4,5-seco-4,5-dioxo-4,5,10(22*H*)-tetrahydropheophorbidate a) [17].

We first converted the easily accessible [18] pheophorbide a methyl ester (**2**) into its Cd^{II} complex, **3**, obtained in 88% yield as a 3:1-mixture of the 13^2-stereoisomers **3**/*β*-**3**

Scheme 2



(= 13^2-epi-**3**)[3]). The Cd^{II} complex **3**/β-**3** was photooxygenated at low temperature (in CH_3OH/CH_2Cl_2 1:19, O_2 (1 atm), Na lamp) to give (10,22-dihydro-4,5-dioxo-4,5-secopheophorbidato a methyl ester) cadmium(II) (32% yield at *ca.* 78% conversion) as a 3:1 mixture of the 13^2-stereoisomers **4**/β-**4** (= 13^2-epi-**4**)[3]). The structure of the secoporphinoid Cd^{II} complex **4** and hence the site of the oxygenolytic cleavage of the macrocycle of **2** were established spectroanalytically, most notably by two-dimensional NMR spectroscopy (see *Fig.*)[4]).

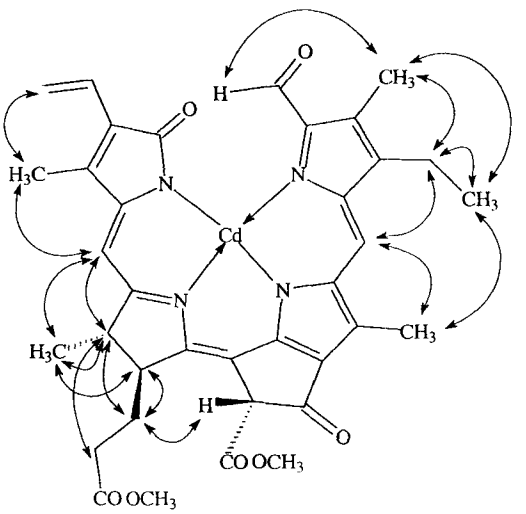


Figure. *Relevant ROESY correlation in the NMR spectrum of* **3** *used for the determination of the site of oxygenolytic cleavage of the tetrapyrrolic macrocycle of* **2** (signal assignments from analysis of COSY, ROESY, HMQC, and HMBC spectra [21])

The secoporphinoid Cd^{II} complex **4**/β-**4** was then reduced with $NaBH_4$ at room temperature and the mixture worked up with dil. HCl to give the 4,5-secopheophorbid ester in 72% yield as a *ca.* 3:1 mixture of the 13^2-stereoisomers **5**/β-**5** (= 13^2-epi-**5**)[3]), from which configurationally uniform **5** was obtained by semipreparative HPLC. The red tetrapyrrole **5** was characterized spectroscopically as the stereoisomer with α configuration at C(13^2) on the basis of a strong NOE between H–C(13^2) and CH_2(17^1) of the side chain at C(17), which, in turn, is bound at the β-face. On the other hand, the minor isomer (β-**5**) was assigned to have β-configuration at C(13^2) based on a strong NOE between H–C(13^2) and H–C(17), rather than, alternatively, to be a geometric isomer of **5** (formed by (15*Z*)/(15*E*) isomerism, as discussed in [20]). Furthermore, **5** was identical with a dimethyl-ester isolate of the red pigment from *Chlorella protothecoides*, when compared by its reported UV/VIS, ^{1}H-NMR, and FAB mass spectra [16].

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[3]) Analysis of the integrals of the signal assigned to H–C(13^2 in the ^{1}H-NMR spectra indicated **3**/β-**3**, **4**/β-**4**, or **5**/β-**5** to be present as *ca.* 3:1 mixtures, **1**/β-**1** as a *ca.* 4:1 mixture of the 13^2-stereoisomers.

[4]) In an analogous photolysis with the corresponding Zn^{II} complex, (pheophorbidato a methyl ester)zinc(II), the major product of photooxidation remarkably was found to be (19,20-dioxo-19,20-secopheophorbidato a methyl ester)zinc(II) (51% yield): the intriguing dependence on the metal of the site of photooxygenolytic cleavage of the chlorin macrocycle parallels that discovered by *Iturraspe* and *Gossauer* [19].

Highly regioselective hydrolysis of the propanoic-ester group of **5**/*β*-**5** was achieved by the use of pig liver esterase. The site of ester hydrolysis was established by NMR spectroscopy (from HMBC spectra [21]). This enzyme-catalyzed ester hydrolysis turned out to occur at a similar apparent[5]) efficiency for the two diastereoisomers. The monoester 10,22-dihydro-4,5-dioxo-4,5-secopheophorbide a was obtained in 98% yield as a *ca.* 4:1 mixture of the two 13^2-stereoisomers **1** and *β*-**1** (13^2-epi-**1**)[3]).

Conclusions and Outlook. – We have reported here, as a first task, the partial synthesis of the 10,22-dihydro-4,5-dioxo-4,5-secopheophorbide a methyl ester (**5**). In this way, we have opened (an alternative) route to the dimethyl ester of the isolation form of the red chlorophyll catabolite from *Chlorella protothecoides* [16] and have provided (classic) support for the structural characterization of this linear tetrapyrrole. The main goal of this work has been the preparation of the monoester **1**, a red bilinone, which may represent a putative intermediary chlorophyll catabolite in senescent chloroplasts [12]. In addition, **1** may also be considered as an intermediate of the degradation of chlorophyll a in the green alga *Chlorella protothecoides*, whose red tetrapyrrolic excrete recently has been suggested to be, in fact, the corresponding diacid [15].

With respect to breakdown of chlorophyll in (some) senescent plants, the intermediary role of **1** appears likely, as *i*) an enzymatic reduction of the C(20)=C(1) bond of the red compound **1** would lead to (yellow) 'fluorescent' chlorophyll catabolites having the constitution of *Bn*-FCC-2 (**II**), which has been isolated from senescent cotyledons of *Brassica napus* [12]; *ii*) the red tetrapyrrole **1** could arise from pheophorbide a by the action of the presumed key enzyme of chlorophyll catabolism in plants, of the putative pheophorbide a oxygenase [23], whose expression in plants is specifically senescence-induced [24]. Experiments are underway to test the hypothetical role of the red tetrapyrrole **1** in chlorophyll breakdown in senescent tissues of green plants [22].

We would like to thank Prof. Dr. *Albert Eschenmoser*, for generously making available a collection of chlorophyll derivatives, *Sebastian Geiger* for helpful exploratory photooxygenation experiments, and Univ.-Doz. Dr. *K.-H. Ongania* for measuring the FAB mass spectra. We are indebted to the *Stipendien-Fonds des Verbandes der Chemischen Industrie* (Frankfurt, Germany) for a post-doctoral stipendium and to the *Austrian National Science Foundation* (*FWF*) for a *Lise Meitner* fellowship to *B.G.*

Experimental Part

General. Reagents used were reagent-grade commercials. HPLC solvents and pig liver esterase (EEC 23277737, 215 U/mg) were from *Fluka*, Buchs, Switzerland. TLC: *Polygram SIL G/UV254* and *Polygram ALOX N/UV254* both from *Macherey-Nagel*. Column chromatography (CC). Silica gel *60* (0.040–0.063 mm) from *Merck*. HP-TLC: *RP-18* plates from *Merck*. HPLC: *Phenomenex*, *ODS-Hypersil* 5μ, 250 × 4.6 mm i.d., *Gynkotek* 'high precision pump' *480G* with vacuum on-line degasser, *Gynkotek* diode array detector *DA340*, UV/VIS detection between 190 and 600 nm; all chromatograms were taken at r.t. UV/VIS Spectra: *Hitachi-U3000* spectrophotometer, λ_{max} [nm] (log ε). CD Spectra: *JASCO J715* spectropolarimeter, [nm] (rel. $\Delta\varepsilon$). FT-IR (0.2%

[5]) The enzyme-catalyzed hydrolysis (at pH 7.9) of isomerically pure **5** and workup similarly gave a 4:1 mixture of **1**/*β*-**1**. Related observation concerning the configurational lability at $C(13^2)$, have been noted elsewhere, *e.g.*, for **5**/*β*-**5** [15]. According to exploratory HPLC experiments, separation of the epimer mixture **1**/*β*-**1** appears feasible (*e.g.*, for analytical purposes), but (so far) has not been scaled up for preparative experiments, as configurational equilibration at $C(13^2)$ is expected under the conditions of enzymatic incubation experiments [22].

in KBr): *Mattson 3000*; ν [cm^{-1}]. NMR Spectra: *Varian unity$_{plus}$500* (500 MHz, 26°), *Bruker AM300* (300 MHz, 23°; δ [ppm], J in Hz; in $CDCl_3$ ($\delta(CHCl_3) = 7.24$ (^{1}H) and 7.70 ppm (^{12}C)) and CD_3OD ($\delta(CHD_2OD) = 3.39$ ppm (^{1}H); FAB-MS: *Finnigan MAT 95-S* (Cs^+ bombardment at 20 keV, 2 μA); positive-ion mode; matrix 3-nitrobenzyl alcohol; in *m/z* (%).

Pheophorbide a Methyl Ester (**2**) was prepared according to [18a]. The identification of **2** was achieved by comparison of the spectroscopic data of **2** with those in [18b].

(Pheophorbidate a Methyl Ester)cadmium(II) (**3**/β-**3**). In 20 ml of freshly distilled CH_2Cl_2 80 mg (0.13 mmol) of stereochemically pure, crystalline **2** [18] were dissolved. A suspension of (acetylacetonato)cadmium (400 mg in 20 ml of MeOH) was added and this mixture stirred at 40° for 1 h under N_2 in the dark. After cooling to r.t., the mixture was filtered through a short (50 × 20 mm) column filled with Al_2O_3 and the eluate evaporated. The residue was dissolved in 2 ml of CH_2Cl_2 and was precipitated by dropping this soln. into 30 ml of hexane. The mixture was centrifuged (4000 U/min 5 min) and the supernatant discarded. The solid residue was dried to give 83 mg of blue-green **3**/β-**3** (3:1)[3]. UV/VIS (CH_2Cl_2): 340 (sh, 4.38), 380 (sh, 4.55), 412 (sh, 4.81), 431 (4.87), 530 (3.55), 614 (4.04), 661 (4.73). IR: 2955*m*, 2924*m*, 2860*m*, 1734*s*, 1689*s*, 1584*m*, 1535*s*, *etc.* ^{1}H-NMR (**3**; 500 MHz, $CDCl_3$): 0.98 (*t*, $J = 7.6$, Me(8^2)); 2.09 (*d*, $J = 7.2$, Me(18^1)); 2.24 (*s*, Me(7^1)); 2.30 (*m*, H_a–C(17^1)); 2.70–2.90 (*m*, H_b–C(17^1), CH_2(17^2), CH_2(8^1)); 3.22 (*s*, Me(2^1)); 3.32 (*s*, Me(12^1)); 3.55 (*s*, Me(17^5)); 4.09 (*s*, Me(13^5)); 4.21 (*m*, H–C(17)); 4.32 (*qd*, $J = 7.2$, 3.0, H–C(18)); 5.90 (*dd*, $J = 11.5$, 1.5, H_{cis}–C(3^2)); 6.00 (*dd*, $J = 17.5$, 1.5, H_{trans}–C(2^2)); 6.10 (*s*, H–C(13^2)); 7.54 (*dd*, $J = 17.5$, 11.5, H–C(3^1)); 7.58 (*s*, H–C(5)); 7.88 (*s*, H–C(10)); 8.10 (*s*, H–C(20)). ^{13}C-NMR (**3**; 75 MHz, $CDCl_3$): 10.1(C(7^1)); 12.1(C(2^1)); 12.3(C(12^1)); 16.6(C(8^2)); 18.4(C(8^1)); 23.8(C(18^1)); 29.2(C(17^1)); 31.0(C(17^2)); 49.8(C(18)); 51.3(C(1)); 51.7(C(17^5)); 52.8(C(13^5)); 65.4(C(13^2)); 91.8(C(20)); 98.0(C(5)); 104.5(C(15)); 105.5(C(10)); 120.1(C(3^2)); 129.5(C(3^1)); 129.6(C(12)); 131.5(C(7)); 135.2(C(2)); 137.0(C(11)); 138.7(C(3)); 141.4(C(8)); 143.5(C(3)); 146.4(C(9)); 146.7(C(13)); 149.9(C(6)); 153.8(C(1)); 157.1(C(16)); 161.0(C(14)); 168.0(C(19)); 170.50(C(13^2)); 173.3(C(17^3)); 189.9(C(13^1)). FAB-MS: 720.8 (29.7), 719.8 (52.1), 718.1 (97.1, $C_{36}H_{36}Cd_1N_4O_5^+$, M^+), 717.8 (78.8), 716.8 (81.6), 715.8 (55.8), 714.7 (33.9).

(10,22-Dihydro-4,5-dioxo-4,5-secopheophorbidato a Methyl Ester)cadmium(II) (**4**/β-**4**). A soln. of **3**/β-**3** (30 mg in 100 ml of CH_2Cl_2/MeOH 95:5) was transferred to a three-neck flask supplied with a thermometer, a reflux condenser, and a fritted gas-dispersion tube and cooled down to −40°. O_2 was bubbled through the cooled soln. and the flask irradiated with a Na vapor lamp at a distance of 10 cm. After 1 h, the mixture was transferred into a separating funnel and was shaken with K_3PO_4 (100 mM, pH 7), filtered through a plug of cotton wool, and evaporated. The product was purified by CC (silica gel *60* (*Merck*, 40–60 μ) at −20° with CH_2Cl_2/CH_3OH 95:5). The brownish fraction of **4**/β-**4** was then washed with K_3PO_4 (100 mM, pH 7), filtered through a plug of cotton wool, and evaporated. Reprecipitation from $CHCl_3$/hexane and drying under high vacuum yielded 9.5 mg (32%) of **4**/β-**4** (75:25 ± 5)[3]. UV/VIS (CH_2Cl_2, rel. ε): 318.5 (1.00), 418 (0.90), 452 (0.85); 660 (br., 0.22). ^{1}H-NMR (**4**, 500 MHz, $CDCl_3$), 1.11 (*t*, $J = 7.7$, Me(8^2)); 1.13 (*d*, $J = 7.2$, Me(18^1)); 1.72/1.90 (*m*, CH_2(17^1)); 2.02 (*s*, Me(2^1)); 2.17 (*s*, Me(7^1)); 2.20–2.40 (*m*, CH_2(17^2) superimposed by H–C(17)); 2.40 (*s*, Me(12^1)); 2.50–2.60 (*m*, CH_2(8^1) superimposed by H–C(18)); 3.62 (*s*, Me(17^5)); 3.72 (*s*, Me(13^5)); 4.37 (*s*, H–C(13^2)); 5.22 (*s*, H–C(20)); 5.38 (*d*, $J = 11.5$, H_{cis}–C(3^2)); 6.10 (*d*, $J = 18.2$, H_{trans}–C(3^2)); 6.39 (*dd*, $J = 18.2$, 11.5, H–C(1^1)); 7.03 (*s*, H–C(10)); 9.70 (*s*, H–C(5)). ^{13}C-NMR (**4**, from 2D-correlation spectra, 125 MHz, $CDCl_3$): 9.3(C(7^1)); 10.1(C(2^1)); 11.4(C(12^1)); 16.4(C(8^2)); 17.9(C(8^1)); 19.7(C(18^1)); 28.1(C(17^1)); 31.3(C(17^2)); 46.8(C(17)); 49.2(C(18)); 52.3(C(17^5)); 52.8(C(13^5)); 62.2(C(13^2)); 94.2(C(20)); 113.2(C(15)); 122.0(C(3^2)); 126.6(C(3^1)); 129.4(C(7)); 129.4(C(10)); 131.0(C(12)); 131.5(C(3)); 143.4(C(1)); 144.4(C(9)); 147.2(C(8)); 151.8(C(13)); 153.1(C(6)); 164.4(C(2)); 169.0(C(13^3)); 173.2(C(17^3)); 184.4(C(19)); 186.3(C(5)); 187.5(C(13^1)). FAB-MS: 753.2 (30), 752.2 (38), 751.0 (100, $C_{36}H_{37}Cd_1N_4O_7^+$, $[M + 1]^+$), 750.0 (86), 749.0 (77), 748.0 (54), 639.5 (73, $C_{36}H_{39}N_4O_7$, $[M + 3 - Cd]^+$). HR-FAB-MS: 751.1696 ± 0.015 mmU; calc. 751.1696 for $C_{36}H_{37}Cd_1N_4O_7^+$.

10,22-Dihydro-4,5-dioxo-4,5-secopheophorbide a Methyl Ester (**5**/β-**5**): To a soln. of **4**/β-**4** (4.2 mg) in 12 ml of THF/$CHCl_3$ 10:2, 2 mg of $NaBH_4$ were added with stirring. Immediately after the color of the suspension had changed from brownish to blue (*ca.* 2 min after the addition of $NaBH_4$), the mixture was poured into 0.03M HCl, whereupon its color rapidly changed from blue to red. It was extracted with *ca.* 20 ml of CH_2Cl_2, the org. layer washed with K_3PO_4 (100 mM, pH 7), filtered through a plug of cotton wool, and evaporated. The crude **5** was purified by HP-TLC with MeOH/MeCN 95:5. The red zone was extracted twice with MeOH and dried under high vacuum: 2.9 mg (72%) of **5**/β-**5** (*ca.* 3:1). Separation of **5**/β-**5** was achieved by semiprep. HPLC (*ODS* column (*Seibersdorf*, 250 × 4.6 mm), linear gradient MeOH/H_2O 70:30 → MeOH): t_R(**5**) 13.7 (**5**) and t_R(β-**5**) 14.1 min (β-**5**). The fraction containing **5** was collected and submitted to spectroscopic analysis. UV/VIS (MeOH, rel. ε): 271.0 (0.78), 316.3 (1.00), 423 (sh, 0.34), 455.0 (0.45), 491.5 (0.46), 532.0 (0.43), 577.0 (0.27). CD (MeOH): 240.5 (0.57), 267.5 (0.62), 311.5 (0.47), 374 (−0.23), 452 (−0.10), 484 (−0.12), 524 (−0.08). ^{1}H-NMR (500 MHz,

$CDCl_3$): 0.94 (*t*, *J* = 7.6, Me(8^2)); 1.17 (*d*, *J* = 7.0, Me(18^1)); 1.71/1.95 (*m*, $CH_2(17^1)$); 2.15 (*s*, Me(2^1)); 2.22 (*s*, Me(7^1)); 2.26 (*m*, $CH_2(17^2)$), superimposed by 2.25 (*s*, Me(12^1)); 2.36 (*q*, *J* = 7.5, $CH_2(8^1)$); 2.51 (*m*, H–C(17)); 2.72 (*q*, *J* = 7.0, H–C(18)); 3.64 (*s*, Me(17^5)); 3.74 (*s*, Me(13^5)); 3.94 (*s*, $CH_2(10)$); 4.41 (*s*, H–C(13^2)); 5.67 (*d*, *J* = 11.8, H_{cis}–C(3^2)); 5.67 (*s*, H–C(20)); 6.48 (*d*, *J* = 17.7, H_{trans}–C(3^2)); 6.61 (*dd*, *J* = 17.7, 11.8, H–C(3^1)); 8.63 (br., HN); 9.46 (*s*, H–C(5)); 9.88 (br., HN); 10.09 (br., HN). ^{13}C-NMR (from 2D-correlation spectra, 125 MHz, $CDCl_3$): 8.9(C(7^1)); 9.1(C(12^1)); 9.5(C(2^1)); 15.0(C(8^2)); 17.0(C(8^1)); 18.8(C(^{1})); 23.2(C(10)); 28.5(C(17^1)); 31.0(C(17^2)); 46.6(C(17)); 50.3(C(18)); 51.7(C(17^5)); 52.8(C(13^5)); 60.7(C(13^2)); 98.0(C(20)); 112.3(C(12)); 115.4(C(15)); 123.2(C(3^2)); 124.9(C(8)); 125.3(C(3^1)); 127.4(C(13)); 128.4(C(6)); 129.6(C(3)); 131.4(C(7)); 133.4(C(9)); 135.4(C(11)); 139.6(C(1)); 147.3(C(2)); 168.8(C(13^2)); 173.4(C(17^3)); 177.1(C(5)); 178.8(C(19)); 186.1(C(13^1)). FAB-MS: 642.5 (41), 642.5 (100, $C_{36}H_{41}N_4O_7^+$, $[M + 1]^+$), 640.5 (72), 639.5 (37). HR-FAB-MS: 641.3044 ± 0.010; calc. 641.2975 for $C_{36}H_{41}N_4O_7$.

10,22-Dihydro-4,5-dioxo-4,5-secopheophorbide a Methyl Ester (**1**). A soln. of 3 mg of **5**/*β*-**5** in 0.5 ml of DMSO was added dropwise into a stirred soln. of 3 mg of pig liver esterase in 10 ml of 0.25M K_3PO_4 puffer (pH 7.9) thermostated to 38°. The reaction mixture was stirred for 13 h in the dark and then poured into 15 ml of AcOMe. This mixture was treated with 20 ml of 2% aq. citric acid and the mixture shaken vigorously. The org. layer was isolated, diluted with 15 ml of CH_2Cl_2, and filtered through dry cotton wool. After evaporation, the residue was precipitated from CH_2Cl_2/hexane and dried at high vacuum: 2.8 mg (98%) of **1**/*β*-**1** (4:1). UV/VIS (CH_2Cl_2 rel. *ε*): 271.0 (78), 315.3 (100), 495.5 (38), 535.5 (40), 574.0 (35). ^{1}H-NMR (500 MHz in $CDCl_3$): 0.98 (*t*, *J* = 7.5, Me(8^2)); 1.15 (*d*, *J* = 7.0, Me(18^1)); 1.65 (*m*, 1H, $CH_2(17^1)$); 1.83 (*m*, 1H, $CH_2(17^1)$); 2.09 (*s*, Me(2^1)); 2.19 (*s*, Me($C7^1$)); 2.25 (*m*, $CH_2(17^2)$); 2.25 (*s*, Me(12^1)); 2.39 (*q*, *J* = 7.5, $CH_2(8^1)$); 2.42 (*m*, H–C(17)); 2.69 (*q*, *J* = 7.0, H–C(18)); 3.70 (*s*, Me(13^5)); 3.85/3.95 (*AB*, J_{AB} = 16, $CH_2(10)$); 4.39 (*s*, H–C(13^2)); 5.44 (*d*, *J* = 10.5, H_{cis}–C(3^2)); 5.68 (*s*, H–C(20)); 6.18 (*d*, *J* = 17.5, H_{trans}–C(3^2)); 6.35 (*dd*, *J* = 10.5, 17.5 Hz, H–C(3^1)); 8.80–10.45 (br., HN); 9.32 (*s*, H–C(5)); 10.01–10.45 (br., 2 HN). ^{13}C-NMR (500 MHz, $CDCl_3$): 9.0(C(7^1)); 9.0(C(12^1)); 9.4(C(2^1)); 15.2(C(8^2)); 19.0(C(8^1)); 23.1(C(10)); 28.3(C(17^1)); 30.9(C(17^2)); 46.8(C(17)); 50.2(C(18)); 52.9(C(13^5)); 61.8(C(13^2)); 98.8(C(20)); 112.5(C(12)); 115.8(C(15)); 122.6(C(3^2)); 125.2(C(8)); 125.4(C(3^1)); 128.4(C(6)); 128.6(C(13)); 129.3(C(3)); 132.6(C(7)); 135.0(C(9)); 135.1(C(11)); 140.1(C(1)); 197.2(C(2)); 169.0(C(13^3)); 176.0(C(17^4)); 176.8(C(5)); 178.4(C(19)); 186.7(C(13^1)). FAB-MS: 628.6 (41), 627.6 (100, $C_{35}H_{38}N_4O_7^+$, $[M + 1]^+$), 626.6 (68).

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