A pink colored dioxobilin-type phyllobilin from breakdown of chlorophyll

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Abstract

In senescent leaves of higher plants, colorless chlorophyll (Chl) catabolites typically accumulate that belong to one of two major types, classified as formyloxobilin-type or dioxobilin-type phyllobilins (PBs). Natural yellow and pink colored formyloxobilin-type PBs are also known, named YCCs and PiCCs, respectively. A dioxobilin-type yellow Chl catabolite (DYCC) was recently prepared by partial synthesis, characterized by spectroscopic means and also identified in grapevine leaves. Here, we report the first dioxobilin-type pink Chl catabolite (DPiCC) from its partial synthesis from the DYCC. The DPiCC had similar structural features and absorption characteristics (in the visible spectral region) as the natural PiCC, its formyloxobilin-type analogon. In contrast to the effectively racemic PiCC, the DPiCC is an optically active pigment with a stable new asymmetric carbon center.

Graphical abstract

Keywords Heterocycle · Natural product · Pigment · Porphyrins · Linear tetrapyrroles

Dedicated to Professor Heinz Falk on the occasion of his 80th birthday.

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Introduction

The disappearance of chlorophyll (Chl) in fall leaves and the concomitant development of fall colors have been a fascinating puzzle, unsolved until about 30 years ago [1–3]. Bilin-type Chl catabolites have been discovered and studied in higher plants, classified as phyllobilins (PBs) [4, 5]. Colorless PBs accumulate in senescent leaves as products of the largely common ‘PAO/phyllobilin’ pathway of Chl breakdown [6–8]. They derive from a ‘red Chl catabolite’ (RCC) [9–11], the cryptic red formyloxobilin that results from specific oxygenolysis of the macrocycle of pheophorbide *a* (Pheide *a*) by Pheide *a* oxygenase (PAO), as the common progenitor of the PBs (see Fig. 1) [12, 13]. Enzymatic reduction of RCC by RCC reductases (RCCRs) [7, 8, 14–17] produces blue fluorescent formyloxobilins, named ‘primary

Fig. 1 Structural outline of key steps of the PAO/phyllobilin pathway of Chl breakdown. Oxygenolysis of Pheide *a* with PAO furnishes the red bilin-type tetrapyrrole RCC, which is reduced to one of the two C16-epimeric 'primary' FCCs (*p*FCCs). Further metabolism of *p*FCCs produces two major types of colorless 'nonfluorescent' phyllobilins (PBs), either 1-formyl-19-oxobilanes (NCCs) or 1,19-dioxobilanes (DNCCs), also classified as type-I or type-II PBs, respectively

fluorescent' Chl catabolites (*p*FCCs) [18, 19], from which a variety of fluorescent Chl catabolites (FCCs) are generated by further modifications by the action of chloroplastic or cytosolic enzymes [5, 8, 20, 21]. The typical FCCs are only fleetingly existent and isomerize rapidly in a weakly acidic aqueous environment to corresponding formyloxobilin-type 'nonfluorescent' Chl catabolites (NCCs) [22, 23]. A competitive oxidative FCC deformylation by a cytochrome P450 type enzyme (identified as CYP89A9 in *Arabidopsis thaliana*) leads to corresponding dioxobilin-type fluorescent

Chl catabolites (dioxobilin-type FCCs or DFCCs) [24, 25]. As do FCCs, DFCCs also isomerize stereo-selectively, furnishing their nonfluorescent isomers, dioxobilin type NCCs (DNCCs) [25]. DNCCs, detected as apparently exotic 'urobilinogenoidic' Chl catabolites [26], have meanwhile been shown to be abundant in a variety of leaves and fruit [5, 21, 27–29]. Thus, two major lines of colorless, 'nonfluorescent' Chl catabolites are known to accumulate in senescent leaves of angiosperms, representing either NCCs (type-I phyllobilanes) or DNCCs (type-II phyllobilanes) [4, 5]. Yellow

descendants of NCCs, yellow (formyloxobilin-type) Chl catabolites (YCCs) [30, 31] and analogous pink colored Chl catabolites (PiCCs) [32] have been discovered in extracts of senescent leaves and their structures and reactivity have been investigated [5, 33].

The dioxobilin-type NCCs, named DNCCs [5, 21, 24], have been observed recently in some leaves (and leaf extracts), to be oxidized regio-selectively, similar to NCCs, generating dioxobilin-type yellow Chl catabolites (DYCCs) [34]. Furthermore, the availability of the semisynthetic DYCC **1** (a type-II phylloxanthobilin) invited the exploration of a partial synthesis of its pink colored analog, the DPiCC **2**, a type-II phylloroseobilin (PrB). As shown here, the DYCC **1** did undergo air oxidation readily and selectively, in particular in the presence of Zn(II)-ions, providing the DPiCC **2** (a 1,19-dioxo-1,4,16,19-tetrahydro-phyllobiladiene-*b,c*, according to the semi-systematic phyllobilin nomenclature [4]) and a first representative of the optically active class of the pink colored type-II PrBs.

Results and discussion

For the partial synthesis of DPiCC **2** from DYCC **1** (Fig. 2), we used the method developed for the preparation of the PiCC **3** from YCC [33, 35] via an intermediate blue Zn-complex. When a solution of 7.2 mg of DYCC **1** (prepared from

Vv-DNCC-51 [21]) [34] in DMF was treated with an excess of Zn(OAc)₂ at room temperature and in the presence of O₂, the color of the reaction mixture changed slowly from yellow to blue. After 18 h, a TLC showed complete disappearance of the DYCC **1** and the blue reaction mixture was worked up (Experimental Section). To the solution of the blue residue (tentatively, the Zn-complex of DPiCC) in MeCN, an acidic potassium phosphate buffer was added, leading to a rapid color change of the solution to red, due to the formation of the metal-free DPiCC **2**. Upon purification by desalting on a Sep-Pak Cartridge, 6.6 mg of **2** (92% yield) was isolated as a dark red powder, which was characterized directly by a set of UV/Vis, CD, mass and NMR spectra.

In the absorption spectra of both DPiCC **2** and PiCC **3** [33] in MeOH, the common PrB-chromophore dominated the absorption at long wavelengths (Fig. 3, top). The long wavelength absorption band of both PrBs (**2** and **3**) featured an intense peak with a maximum at 522 nm, accompanied by a shoulder near 485 nm. The extension of the π -system, from two pyrrolic rings in the DYCC **1** to three pyrrolic rings in DPiCC **2**, leads to a bathochromic shift of about 100 nm (to 522 nm) [21, 34]. As was the case for DYCCs, the ring A lactam structure of DPiCC **2**, instead of a formyl pyrrole unit in PiCC **3**, diminished the absorbance near 320 nm, and two less intense maxima at 315 and 338 nm are observed in the spectrum of **2**. The CD spectrum of the (racemic) PiCC **3** in MeOH was

Fig. 2 Left and center: Structural outline of a partial synthesis of the dioxobilin-type pink Chl catabolite DPiCC **2** by air oxidation of the DYCC **1** via a blue intermediate Zn-complex. DYCC **1** is available from oxidation of the natural Vv-DNCC-51 [34]. Right: The structural formula of the formyloxobilin-type pink Chl catabolite PiCC **3** is shown for comparison

Fig. 3 Top: UV/Vis spectra of DPiCC **2** and PiCC **3** in MeOH (1.53×10^{-5} mol dm $^{-3}$); Bottom: CD spectra of DPiCC **2** and PiCC **3** in MeOH (3.1×10^{-5} mol dm $^{-3}$)

featureless, but a pronounced and structured CD spectrum was observed for the solution of the DPiCC **2** (Fig. 3, bottom). Solutions of **2** in MeOH show a weak cotton effect at 336 nm and 285 nm, consistent with the stable configuration of the asymmetric methine HC4. The configuration of C4 of **2** was tentatively assigned as *R*, as it is inherited

intact from the type-II precursors DYCC **1** [34] and Vv-DNCC-51 [21].

The molecular formula of DPiCC **2** was deduced as C₃₄H₃₆N₄O₈, from its ESI-MS spectrum that exhibited strong pseudo-molecular ions at $m/z = 689.1$ [M-H + Na + K]⁺, at $m/z = 667.1$ [M + K]⁺, and at $m/z = 651.4$ [M + Na]⁺ (Fig. S5a-S5c), confirming the (formal) loss of one C, when going from the (formyloxobilin-type) PiCC **3** to the (dioxobilin-type) DPiCC **2**.

In a 500 MHz ¹H NMR spectrum of **2** in CD₃OD, the signals of 30 non-exchangeable protons were observed (Fig. 4). The structure of **2** was established by correlations in two-dimensional ¹H, ¹H-COSY, ¹H, ¹H-ROESY, ¹H, ¹³C-HSQC, and ¹H, ¹³C-HMBC spectra. The signal pattern of a vinyl group and a singlet (vinyl HC15) was observed at intermediate field. The four H₃C groups of **2** were observed at high field as three (apparent) singlets and a pair of similarly intense singlets at 1.71 and 1.79 ppm (of H₃C2¹), consistent with the presence of two similarly important C8²-epimers. The partially saturated lactam unit in **2** leads to a new spin system ABX of the H₂C5-HC4 section (in **3** H₂C5 produced an AB system) (Fig. S2). A complex AB-type system at 2.79 ppm (H_A) and 3.40 ppm (H_B) correlated with a carbon with resonance at 29.6 ppm in HSQC spectrum and was assigned to the methylene group H₂C5. In addition, H_A and H_B coupled with a signal at 4.59 ppm ($J_{AX} = 8.9$ Hz, $J_{BX} = 4.6$ Hz), which was assigned to the asymmetric HC4. Two ¹H, ¹H-ROESY-correlations with similar strengths from HC15 to H₃C17¹ and to H₃C13¹ indicated the *Z*-configuration of the C15=C16 bond. However, as the signals of all HNs and of C8² were lacking in the spectrum in CD₃OD, the configuration at the C10=C11 bond could not be deduced.

To find the signals of exchange-labile protons, DMSO-*d*₆ and CD₃CN were employed as alternative solvents for NMR analyses (Figs. 5, S4 and Table S1). In both solvents, two more NHs' signals were observed at low field. In the spectrum of **2** in DMSO-*d*₆, a signal at 10 ppm was assigned to HN24 of ring D, owing to its correlations in a ¹H, ¹³C-HMBC spectrum to the carbons at ring D. In the ¹H, ¹H-ROESY spectrum HN24 also coupled with the methyl ester signal at 3.64 ppm and with HC8² at 5.05 ppm, indicative

Fig. 4 500 MHz ¹H NMR spectrum of DPiCC **2** in CD₃OD (25 °C, 3.5×10^{-3} mol dm $^{-3}$)

Fig. 5 NMR chemical shift data of DPiCC **2** (500 MHz NMR, 25 °C) depicted in a graphical representation of the derived molecular structure (top: spectrum in CD₃OD; bottom: spectrum in DMSO-*d*₆) Left: Homonuclear correlations from ¹H, ¹H-ROESY experiments (arrows; bold bonds represent spin systems derived from ¹H, ¹H-COSY spectra). Right: Heteronuclear correlations and signal assignments obtained from ¹H, ¹³C-HSQC spectra (shaded boxes) and ¹H, ¹³C-HMBC experiments (arrows). Dashed arrows indicate weak couplings

of *E*-configuration of the C10=C11 double bond, as first deduced for the type-I analog PiCC **3** [32, 35].

Interestingly, an NH signal around 8.1 ppm, which correlated with HC4 at 4.37 ppm, was split into two singlets and could be assigned to HN21 of ring A. The signals of the two NHs', of HC8², H₃C2¹ and H₃C7¹ in DMSO-*d*₆ were, likewise, split into two, indicating the existence of **2** in two nearly equally populated diastereo-isomers. The deduced structure indicates two asymmetric carbon centers in a molecule of the DPiCC **2**, one of which (at C8²) undergoes

epimerization readily, as was observed for PiCC **3** [33]. The stable configuration at C4 of DYCC **1**, provisionally deduced as *R*, and the deduced presence of both DPiCC 8²-epimers (probably as 4*R*,8²*R*- and 4*R*,8²*S*-epimers) gave rise to the two sets of signals.

In CD₃CN, the signals of the two NHs', of H₃C2¹ and of HC8² of **2** also split into two singlets with approximately equal intensity. In the ¹H, ¹H-ROESY spectrum of **2** in CD₃CN, significant correlations of HN21 (at 6.84 ppm) to HC4 (at 4.55 ppm) were observed. The signal of HN21 of

2 underwent a large solvent-induced shift to lower field in DMSO- d_6 , when compared to CD₃CN. This difference may be due to the presence of an H-bond between HN21 of **2** and DMSO, and its absence in CD₃CN. The remaining proton signals of **2** featured similar chemical shifts in both solvents. Except for the protons at their ring A, the signals of DPiCC **2** and PiCC **3** occurred at very similar chemical shift in ¹H NMR spectra taken in DMSO- d_6 (Fig. S3 and Table S1).

As deduced here for DPiCC **2**, pink colored type-II phylloseobilins (PrBs) feature the same chromophore as the type-I analogs (such as PiCC **3**) that extends over three conjugated pyrrole rings and displays a 10*E*,15*Z* double bond configuration. Three N-atoms that are part of the chromophore are ready for coordination to metals, but require a change of the configuration of the C10=C11 double bond to coordinate a metal ion in a tridentate fashion. Indeed, the type-I PrBs undergo the critical double-bond isomerization readily and show remarkable affinity for transition metal ions, such as Ni(II), Cu(II), Zn(II), Cd(II), relevant in the biosphere [33, 35]. Hence, PiCCs (such as **3**) are able to signal the presence of transition metals in aqueous solution, by coordinating them in complexes that exhibit characteristically changed colors and fluorescence properties [33]. However, the chiroptical properties of type-I and type-II PrBs differ characteristically, as only the latter (such as DPiCC **2**) are inherently optically active due to the stable configuration of its asymmetric C4. Type-II PrBs are, thus, expected to show a pronounced capacity for the chelation of transition metals in complexes that are optically active. The ready availability of the DPiCC **2** has meanwhile provided the basis for first studies of the chelation of transition metals by this type-II phylloseobilin and of the properties of the corresponding metal complexes, a subject to be reported in due course. Indeed, their abilities to coordinate metal ions [35, 36] may give phyllochromobilins a role in biological transport, detoxification or activation of transition metals.

The ubiquitous colorless ‘nonfluorescent’ bilane-type Chl catabolites are readily oxidized by a still ill-characterized process in leaves (and leaf extracts), furnishing yellow Chl catabolites (YCCs and DYCCs). These yellow pigments feature the same extended chromophore as available (twice) in the heme-derived bilirubin (BR) and exhibit interesting photochemistry (Z/E photoisomerization [30, 37] and/or [2 + 2] photo-dimerization [31, 37]), relating them to BR, as well [38]. Interestingly, the phylloxanthobilins (PxBs) are easily oxidized further to the pink colored phylloseobilins (PrBs), some of which have also been detected in plant extracts [32]. Possibly, the sequential oxidation to yellow and pink phyllochromobilins represents a route for the further breakdown of Chl beyond the stage of the bilane-type NCCs and DNCCs [5]. Remarkably, colorless tetrapyrrolic Chl catabolites in senescent leaves of fern (*Pteridium aquilinum*) feature a rearranged carbon skeleton at their ring B

[39] that would inhibit their simple oxidation with generation of a type of PrB.

Conclusion

The unique structural features of phyllobilins, as abundant products of Chl breakdown, encourage considering their potential roles in nature. In earlier investigations of Chl breakdown, the then known colorless non-fluorescent Chl catabolites (NCCs) were suggested to represent the ‘final’ products of a (mere) Chl detoxification process [40, 41]. However, the formation in some leaves and fruit of a variety of specifically ‘sugar-decorated’ colorless and blue fluorescent phyllobilins [20, 21], as well as the natural existence of (brightly colored) phyllochromobilins [5, 42], has invited consideration of the critical physiological effects for the natural PBs. Indeed, the enzymatic transformation of the first formed formylxobilin-type (or type-I) PBs to 1,19-dioxobilin-type linear tetrapyrroles (the type-II phyllobilins) [24] generates PBs with an even closer general structural similarity to the heme-derived (hemo-) bilins [4, 43], linear tetrapyrroles with a range of crucial functions in life [38, 43]. The structural relationship may be most pronounced with the colored representatives, the (type-II) phyllochromobilins. The chromophore of the pink PrBs is very similar to the one of phycoviolobilin [43] (its protein-bound Cys-addition form basically is a Z/E-isomer of **2**) [4] and relates also to other heme-derived linear tetrapyrroles important in biological photo-regulation [44–46]. The structural similarities between members of the two large classes of natural linear tetrapyrroles, as well as the major structural difference remaining (the ring E moiety of the PBs, which is absent in the heme-derived bilins [43]), invite studies of the physiological effects of phyllochromobilins in photosynthesizing organism and animals.

Experimental

DMF, pyridine, and CH₂Cl₂ were reagent-grade commercials and distilled before use. MeOH (HPLC grade), from VMR (Leuven, Belgium); water, from Millipore S. A. S. Milli-Q Academic system (18.2 MΩ cm, Molsheim, France); ACS reagent KH₂PO₄ and K₂HPO₄ from Sigma-Aldrich (Steinheim, Germany). Thin-layer chromatography (TLC): Merck 0.25 mm silicagel 60 plates. Sep-Pak-C18 Cartridges (Silica-based bonded phase with strong hydrophobicity), from Waters Associates (Milford, USA). pH Values, measured with a WTW Sentix 21 electrode, WTW pH535 digital pH meter. DYCC **1** (8²*S*,10*R*)-1,19-dioxo-1,4,16,19-tetrahydro-phyllobilene-*c*) [34] was obtained by oxidation of Vv-DNCC-51 (8²*S*,10*R*,

16*epi*)-1,19-dioxo-1,4,16,19-tetrahydro-phyllobilane), which was isolated from the senescent leaves of grapevine (*Vitis vinifera*) [21].

UV/Vis: Varian Cary 60 spectrophotometer; $\lambda_{\max}$ in nm (log ϵ). CD-spectra: JASCO J-715 spectropolarimeter; $\lambda_{\max}$ and $\lambda_{\min}$ in nm ($\Delta\epsilon$). ^1H NMR: Varian UNITY plus 500; δ in ppm with δ (CHD_2OD) = 3.31 ppm, δ (CHD_2CN) = 1.94 ppm, δ ($\text{CD}_3\text{SOCD}_2\text{H}$) = 2.50 ppm, coupling constant J_{HH} in Hz; ^{13}C NMR: chemical shift values and signal assignments from ^1H , ^{13}C -HSQC and ^1H , ^{13}C -HMBC spectra. ESI-MS [m/z (relative intensity %)] : Finnigan LCQ Classic, ESI source, positive ion-mode, flow rate $2\text{ cm}^3\text{ min}^{-1}$, solvent water/MeOH.

Gynkotek 480G 'high precision pump' with vacuum on-line degasser; Gynkotek DA340 diode array detector; Column: Phenomenex ODS-Hyperclone ($5\text{ }\mu\text{m}$, $250\times 4.6\text{ mm i.d.}$) protected with pre-column a Phenomenex Security Guard C18 $4\times 3\text{ mm i.d.}$; flow rate: $0.5\text{ cm}^3/\text{min}$; all chromatograms were taken at room temperature ($23\text{ }^\circ\text{C}$); solvent A: 50 mM aq. potassium phosphate (pH 7.0), solvent B: MeOH, solvent C: H_2O ; solvent composition (A/B/C): 0-5 min: 60/40/0; 5-15 min: from 60/40/0 to 30/70/0; 15-25 min: from 30/70/0 to 0/100/0; 25-35 min: 0/100/0; 35-37 min: 0/100/0 to 0/90/10; 37-42 min: from 0/90/10 to 60/40/0.

1,19-Dioxo-1,4,16,19-tetrahydrophyllobiladiene-*b,c* (DPiCC, 2, ($\text{C}_{37}\text{H}_{48}\text{N}_4\text{O}_4$)) To the solution of 7.2 mg DYCC 1 ($11.5\text{ }\mu\text{mol}$) in 4.3 cm^3 distilled *N,N*-dimethylformamide (DMF), 10.6 mg $\text{Zn}(\text{OAc})_2$ ($57.9\text{ }\mu\text{mol}$, 5 eq) and 0.43 cm^3 pyridine were added. The reaction mixture was purged with O_2 and stirred at room temperature in the dark for 18 h. The color of the reaction mixture changed slowly from yellow to blue. When no DYCC (yellow spot) was detected on the TLC (silica gel without F254, 90/10 MeOH/ CHCl_3), the dark blue reaction mixture was diluted with 30 cm^3 of H_2O and extracted with CH_2Cl_2 ($3\times 30\text{ cm}^3$). The combined organic phases were filtered through a plug of dry cotton wool and the solvent was evaporated under reduced pressure to give a dark blue residue. The blue residue was re-dissolved in 0.2 cm^3 DMF and diluted with 15 cm^3 of MeCN. To this mixture, 30 cm^3 of 100 mM KH_2PO_4 (pH 4.7) was added. After 3 h, a red reaction mixture was obtained and extracted with CH_2Cl_2 ($3\times 30\text{ cm}^3$). The combined organic phase was dried by filtration through a plug of dry cotton wool and was concentrated under reduced pressure to get the raw product DPiCC 2 as dark red residue. The raw DPiCC 2 was dissolved in 2 cm^3 of MeOH and diluted with 3 cm^3 of 50 mM potassium phosphate buffer (pH 7.0). The solution was loaded on a Sep-Pak-C18 cartridge (5 g). After washing with 20 cm^3 H_2O , the red fraction was washed down with MeOH/ H_2O (3/4, v/v). The obtained red fractions were combined and the MeOH was removed completely under reduced pressure (10 mbar). Then, the solution was frozen in liquid

N_2 and lyophilization was employed to get the product. Finally, 6.6 mg of DPiCC 2 was obtained as dark red powder and yield is 92%. UV/Vis (MeOH, $3.6\times 10^{-4}\text{ mol dm}^{-3}$): $\lambda_{\max}$ (log ϵ) = 522 (4.54), 491sh (4.48), 338 (4.11), 315 (4.12), 211 (4.48) nm ($\text{mol}^{-1}\text{ dm}^3\text{ cm}^{-1}$); UV/Vis (MeCN, $3.1\times 10^{-4}\text{ M}$): $\lambda_{\max}$ (log ϵ) = 526 (4.53), 495sh (4.45), 336 (4.11), 316sh (4.08), 212 (4.43) nm ($\text{mol}^{-1}\text{ dm}^3\text{ cm}^{-1}$); CD (MeOH, $3.6\times 10^{-4}\text{ M}$): $\lambda_{\min/\max}$ ($\Delta\epsilon$) = 336 (− 1.6), 285 (− 1.5) nm (deg $\text{cm}^2\text{ dmol}^{-1}$); CD (MeCN, $3.1\times 10^{-4}\text{ M}$): $\lambda_{\min/\max}$ ($\Delta\epsilon$) = 342 (− 1.7), 277 (− 1.6) nm (deg $\text{cm}^2\text{ dmol}^{-1}$); ^1H NMR (500 MHz, CD_3OD): δ = 1.71/1.79 (two s, in total 3H, H_3C^{21}), 2.14 (s, 3H, H_3C^{131}), 2.19 (s, 3H, H_3C^{71}), 2.22 (s, 3H, H_3C^{171}), 2.42 (m, 2H, H_2C^{122}), 2.65 (m, 1H, $\text{H}_\text{A}\text{C}^{31}$), 2.79 (dd, J = 8.9, 14.3 Hz, 1H, $\text{H}_\text{A}\text{C}^5$), 2.84 (m, 1H, $\text{H}_\text{B}\text{C}^{31}$), 3.12 (m, 2H, H_2C^{121}), 3.40 (dd, J = 4.6, 14.3 Hz, 1H, $\text{H}_\text{A}\text{C}^5$), 3.76 (s, 3H, H_3C^{85}), 3.77 (m, 1H, $\text{H}_\text{A}\text{C}^{32}$), 3.83 (m, 1H, $\text{H}_\text{B}\text{C}^{32}$), 4.59 (m, 1H, HC4), 5.52 (d, J = 11.7 Hz, 1H, $\text{H}_\text{A}\text{C}^{182}$), 6.13 (s, 1H, HC15), 6.33 (dd, J = 1.4, 17.7 Hz, 1H, $\text{H}_\text{B}\text{C}^{182}$), 6.66 (dd, J = 11.7, 17.7 Hz, 1H, HC18¹) ppm; ^{13}C NMR (125 MHz, CD_3OD , obtained on basis of ^1H , ^{13}C -HSQC and ^1H , ^{13}C -HMBC spectra): δ = 8.1 (C^{21}), 9.0 (C^{71} & C^{171}), 9.1 (C^{131}), 24.6 (C^{121}), 29.6 (C^5), 30.6 (C^{31}), 38.6 (C^{122}), 52.8 (C^{85}), 60.3 (C^4), 60.9 (C^{32}), 98.8 (C^{15}), 117.2 (C^7), 121.0 (C^{182}), 126.7 (C^{181}), 128.8 (C^{18}), 130.6 (C^2), 136.8 (C^8), 137.9 (C^{13}), 141.2 (C^{12}), 142.1 (C^{17}), 144.8 (C^6), 145.7 (C^{16}), 147.1 (C^{11}), 155.8 (C^3), 166.4 (C^{14}), 171.2 (C^{83}), 172.0 (C^{19}), 176.1 (C^1), 180.9 (C^{123}) ppm; ESI-MS (positive mode): m/z = 1337.1 (53, [2 M-2H + 3 K]⁺), 1355.1 (41, [2 M-2H + Na + 2 K]⁺), 1334.1 (38), 1333.1 (46, [2 M-H + 2 K]⁺), 1318.2 (35), 1317.2 (50, [2 M-H + K + Na]⁺), 707.2 (40), 706.2 (70), 705.2 (82, [M-H + 2 K]⁺), 691.1 (25), 690.1 (40), 689.1 (100, [M-H + Na + K]⁺), 669.1 (10), 668.1 (25), 667.1 (78, [M + K]⁺), 651.4 (28, [M + Na]⁺).

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