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The Profile of Chlorophyll Catabolites in Senescent Leaves of *Epipremnum aureum* Includes a Catabolite Esterified with Hydroxytyrosol 1-*O*-glucoside

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ABSTRACT: Despite the fact that chlorophyll degradation is a physiological phenomenon occurring daily in all photosynthetic tissues, chlorophyll catabolites are not fully identified. Three new forms (**1**, **3**, and **4**) of linear chlorophyll catabolites (phyllobilins) have been characterized in senescent leaves of *Epipremnum aureum* with spectroscopic data. Compound **1** is a hypermodified blue fluorescent chlorophyll catabolite (*hmFCC*) esterified with the potent antioxidant hydroxytyrosol. The sequestration of this phenol by a chlorophyll catabolite could explain the physiological meaning of the persistence of *hmFCCs* in some senescent plants. Compound **3**, a yellow chlorophyll catabolite (*YCC*) originated from the oxidation at C-15 of **1**. *YCCs* have been identified previously and are exclusively formed in the plant vacuole from the final non-fluorescent chlorophyll catabolites (*NCCs*). The presence of **3** in leaves implies a new reaction in chlorophyll catabolism, as the characterization of **3** implies that *YCCs* can be also be oxidized in the cytosol from *FCCs*. Finally, phyllobilin **4** represents a new type of *YCC* characterized by an inflexible bicyclo glucosyl moiety linked through an intramolecular esterification of the propionic acid residue with the C-3 hydroxy group. The corresponding *NCC* precursor was recently identified and now the characterization of **4** shows that even this rigid structure can be further oxidized. Undoubtedly, the characterization of phyllobilins is essential to completely comprehend chlorophyll degradation.

During the vital senescence period of vegetable tissues, including leaves and fruits, chlorophylls are broken down into linear chlorophyll catabolites, named phyllobilins. These compounds have been shown to develop significant biological functions and actions¹ as antioxidants,² in detoxification routes,³ as signaling compounds for animals,⁴ or as toxins for pathogens.⁵ Although the aim of the catabolism of chlorophylls remains unclear, the general PaO/phyllobilins pathway⁶ is a phenomenon occurring in all photosynthetic organisms, even in the evergreen species, which could be summarized as outlined in Scheme 1: conversion of chlorophyll *b* to chlorophyll *a*; transformation of chlorophyll *a* into pheophorbide *a* by dephytylation and dechelation of the magnesium ligand; oxidative opening of the pheophorbide *a* macrocycle to generate the red chlorophyll catabolite (RCC), and stereospecific reduction of the RCC at the C-15/C-16 double bond to form the primary fluorescent chlorophyll catabolite (*p*FCC).⁷ The term *primary* refers to the absence of modifications in the structure. Subsequently, the *p*FCC could be modified by hydroxylation at C²,³ although the enzyme responsible for this reaction is unknown. Hydroxylation is a pre-requisite for further enzymatic reactions. All these reactions are materialized in the chloroplast, where the multi-enzymatic complex named ‘chlorophyll catabolism enzymes’ performs its action in a tunneling-fashion.⁸ Both types of FCCs are transported to the cytosol where they may remain unchanged or become the substrate for the cytochrome P-450, CYP89A9, which decarbonylates the C-1 formyl group.⁹ Thus, the FCCs are split into two classes, those that keep the formyl group at C-1

hydroxymethylation reaction may take place, and finally the methyl ester function at C-8 could be hydrolyzed or not.¹⁰

Scheme 1. Phyllobilins in senescent leaves of *Epipremnum aureum*.

FCCs/DFCCs have a fleeting existence as they are rapidly transformed and, in fact, most of these structures have only been inferred and not properly identified. FCCs/DFCCs are stored into the vacuole where the acidic pH induces the final nonenzymatic isomerization at C-10-C-11 to the non-fluorescent chlorophyll catabolites (NCCs, type I-phyllobilins)¹¹ or the

NCCs, the yellow chlorophyll catabolite (YCC) has been identified. During the past few years, only five different YCCs originating from the corresponding NCCs have been described.¹³⁻¹⁷

However, an unexpected divergent chlorophyll degradation pathway induces the accumulation in the cytosol of a new type of phyllobilin, *hmFCC* (hypermodified-FCC). This is only possible if the propionic acid residue at C-12 is esterified with a functional group,¹¹ so that the resulting *hmFCC* eludes the final nonenzymatic isomerization in the vacuole, remaining in the cytosol with the FCC configuration. *hmFCCs* (Figure S1, Supporting Information) have been exclusively described in three plant species: *Musa acuminata* (Colla), *Spathiphyllum wallisii* (Regel) and *Vitis vinifera* L.^{5,18-20} These unprecedented blue luminescent compounds have been proposed as deliberately biosynthesized with the biological purpose of generating luminescent pigments in specific organs.⁵

The nomenclature of phyllobilins was established in 1993,²¹ and initially the system was functional. Traditionally, the name is formed with the initials of the plant species in Latin followed by the initials of the type of phyllobilins and ending with a number referring to the polarity of the compound in the HPLC chromatogram. However, due to the increasing number of compounds identified since then, the nomenclature system becomes confusing, as the same compound receives different names depending of the plant source and the number of phyllobilins present in that plant species. As mass spectrometry has turned into a useful tool for phyllobilin characterization,^{16,22-25} an alternative code²³ was proposed. The new systematic

usual chlorophyll catabolism. Therefore, the aim of this work was to describe the chlorophyll catabolite profile in an evergreen species, the leaves of pothos (*Epipremnum aureum*, Linden & André G.S.Bunting), as this species belongs to the Araceae family, as well as the peace lily (*Spatiphyllum wallisii*, Regel), so that the prospect of finding *hmFCCs* increases *a priori*. The characterization of the new *hmFCC* (**1**) with hydroxytyrosol attached, probably explains the persistence of this type of phyllobilin in senescent tissue. Unexpectedly, two new types of oxidized phyllobilins (**3** and **4**) accumulated in the senescent leaves of pothos, revealing new biochemical reactions in the chlorophyll degradation pathway that takes place in plants during senescence. In addition, a new characteristic qualifier product ion has been predicted for the bicyclic phyllobilins (**2** and **4**).

RESULTS AND DISCUSSION

Figure 1 shows the chromatogram trace at 320 nm, where NCCs, FCCs and YCCs display the maximum absorbance, as well as the first screening that is commonly performed to manually locate the initial tentative chlorophyll metabolites. Several peaks with the typical UV-Vis spectrum of chlorophyll catabolites²⁶ were annotated. Some of them showed the characteristic spectrum of NCCs (peaks 2 and 3) with the signal produced by the 1-formyl-pyrrole arrangement at ring A at 320 nm, others (peaks 4, 5, and 6) showed the characteristic absorption maxima of the YCCs (310 nm and 426 nm⁴) and peak 1 displayed the typical maxima corresponding to *hmFCC* products, that is, 320 nm and 360 nm²⁷ (Figure 1 insets). The

Indeed, the picture of a pothos leaf with green and yellow sectors taken with a mounted camera with a black-light filter, displays the presence of blue fluorescence patches (Figure 1 inset).

Figure 1. HPLC chromatogram corresponding to the analysis of phyllobilins from *Epipremnum aureum* detected at 320 nm. Peaks as in Table 1. Insets correspond to the UV-Vis spectrum of compound **1** (FCC), **2** (NCC) and **3** (YCC). The UV-Vis spectrum of **4** is identical to **3**. The black arrows in the picture of a pothos leaf with green and yellow sectors taken with a mounted camera with a black-light filter, indicate the blue fluorescence.

this phyllobilin has not yet been identified. Figure 2 depicts the tandem MS obtained at higher (Figure 2A) and lower (Figure 2B) mass range values of compound **1**, while the structural identification of the product ions (Table 1) was performed with the assistance of the MassFrontierTM software and the expert-curated MS² fragmentation-based reactions for chlorophyll catabolite.²⁴ *hmFCC* products with similar fragmentation yields are *hmFCC_943* (*Ma*-FCC-69 and *Sw*-FCC-62, Figure S3, Supporting Information) described by Vergeiner *et al.*,²⁸ and Kraütler *et al.*,²⁰ respectively. Thus, these references include the fragmentation of ring A and the loss of the 3,4-dihydroxyphenethyl moiety attached to *O*-6 of the β -glucopyranosyl esterifying the C-12 propanoic acid moiety. In addition, **1** generates a further product ion of 162 Da, corresponding to a glucopyranosyl residue (fragmentation 5 in Figure S3, Supporting Information). This glucopyranosyl residue could be located at one of the different free hydroxylated functions in the *hmFCC_943* (*Ma*-FCC-69/*Sw*-FCC-62) (Figure S4, Supporting Information). To infer the position of the glucopyranosyl residue, an *in silico* MS²-fragmentation study was performed with different alternative structural arrangements of the substituents (Figure S4, Supporting Information), and the lists of theoretical product ions were compared for screening the experimental qualifier product ions (Table 1). Thus, only the *in silico* list of one configuration included all the experimental product ions, while the other lists failed to predict two characteristic fragments. Indeed, the product ion observed at *m/z* 341.1228, which arises from the glucopyranosyl-3,4-dihydroxyphenethyl substructure (Figure S4,

characteristic product ions revealed key structural arrangements of the protonated molecular ion, including a methyl ester function at C-8², a ring D with an unsubstituted vinyl group at C-18, the presence of 1-*O*-glucopyranosyl moiety and a dihydroxyphenethyl group (Figure S4, Supporting Information). With this experimental and literature-based information, we propose that **1** observed in senescent leaves of pothos is the glucosylated derivative of *hmFCC_943* (*Ma*-FCC-69/*Sw*-FCC-62) (Figure S4, Supporting Information).

The chromatographic peaks observed at 51.20 min and 52.50 (**2**) min (peaks 2 and 3) showed spectroscopic properties that match with those of NCCs (Figure 1). Peak 2 is featured by a mass spectrum (Figure S2B, Supporting Information) that indicates an elemental composition of C₄₁H₅₁N₄O₁₃ (m/z 807.3417 [M+H]⁺; calcd. for C₄₁H₅₁N₄O₁₃, 807.3447 Da), that fits with the identified NCC in senescent leaves of tobacco, maize, pear, apple, lime tree, banana, plum, lemon, wych elm, and in yellow plum fruits.^{2,10,13,15,17,25,29-31} This NCC product is characterized by a vinyl group at C-18, a glucopyranosyl ester function at C-3² and a methyl ester function at C-8² (Scheme 1). The tandem MS of NCC_807 replicated that of the *Zm*-NCC-2 product from senescent maize leaves, which was used as standard for UV-Vis, mass spectrum and tandem MS comparison. Hence, the product ions correlating with the significant structural arrangements of NCC_807 were confirmed as detailed in Table 1. Additionally, the elemental composition and formulas of the product ions were checked with the SmartFormula3D algorithm,²⁵ increasing the reliability of the identification. Therefore, considering the

Figure 2. Tandem HRMS spectra of compound **1** in senescent leaves of *Epipremnum aureum* acquired at high (A) and lower (B) mass range values. Product ions are: 1, 1087.4386, $[M+H-H_2O]^+$; 2, 1073.4238, $[M+H-MeOH]^+$; 3, 982.3812, $[M+H-ring\ D]^+$; 4, 951.3844, $[M+H-dihydroxyphenethyl]^+$; 5, 943.3916, $[M+H-glucopyranosyl]^+$; 6, 915.3602, $[M+H-C_8H_{13}O_5]^+$; 7, 789.3315, $[M+H-glucopyranosyl-dihydroxyphenethyl]^+$; 8, 753.3129, $[M+H-glucopyranosyl-dihydroxyphenethyl-2\times H_2O]^+$; 9, 636.2453, $[M+H-glucopyranosyl-ring\ A-dihydroxyphenethyl]^+$; 10, 341.1228, $[M+H-C_{30}H_{41}N_4O_{11}-H_2O]^+$. Inset in Figure 2B

catabolite product that shows a bicyclo[17.3.1]glycoside moiety that links through an intramolecular esterification the propionic residue at C-12 with the hydroxy function at C-3² (Scheme 1). In fact, **2** is the product of the cyclization reaction of NCC_807 through an esterification reaction and the consequent loss of water. Recently, the bicyclic FCC precursor of **2** has been identified in senescent leaves of grapevine (FCC_789, *Vv*-FCC-55)⁵. The product ions described for NCC_789 (*Ug*-NCC-53)¹⁰ are m/z 757.20 (loss of MeOH from the methyl ester function) and m/z 666.27 (loss of ring D, Scheme 1), while the product ion corresponding to the sugar moiety fragmentation was absent. We produced *in silico* the theoretical product ions after MS² analysis of **2**, pondering the expert-curated MS² fragmentation-based reactions²⁴ for chlorophyll catabolite products. Thus, the characteristic product ions that contain the structural information to arrange that of the parent protonated molecule were experimentally observed at m/z 757.3081 [M+H-MeOH]⁺; m/z 739.2957 [M+H-MeOH-H₂O]⁺; m/z 715.2975 [M+H-MeOH-HOAc]⁺; m/z 604.2292 [M+H-MeOH-H₂O-ring A]⁺; m/z 442.1764 [M+H-MeOH-ring A-glucopyranosyl]⁺. The elemental composition and formulas of these characteristic product ions (Table 1) were checked with the SmartFormula3D algorithm, with mass error and mSigma values below the tolerance limits. The MS² products of NCC_807 (and the characteristics fragmentations of NCCs) and the MS² spectrum of the rigid bicyclic structure of **2** are rather similar (Table 1). Only the loss of HOAc seems to be characteristic of the rigidized structure of **2**. Consequently, considering the experimental spectrometric

C₅₅H₆₇N₄O₂₀ displaying a protonated molecular ion at m/z 1103.4384 (calcd. for C₅₅H₆₇N₄O₂₀, 1103.4343) (Table 1; Figure S2D, Supporting Information). The acquisition of the tandem MS spectrum revealed some characteristic product ions also featured in the MS analysis of **1** including the loss of a glucopyranosyl unit at m/z 941.3811 and the combined fragmentation of ring A with the glucopyranosyl moiety at C-3², and the glucopyranosyl-dihydroxyphenethyl unit at m/z 490.1973. The consistency of these product ions was checked with the SmartFormula3D algorithm, while their structures were generated *in silico* with the MassFrontierTM software. We propose that **3** (YCC_1103) is the oxidative derivative of **1** (*hm*-FCC_1105) (Scheme 1). The accumulation of **3** implies an unprecedented reaction as it was supposed that *hm*FCCs do not undergo further catabolic processes and accumulate intact in the cytosol of senescent tissues.³¹ However, the unravelling of **3** shows that *hm*FCC catabolites could be oxidized to YCCs in the same fashion as the NCCs.^{4,32}

The next assumed YCC compound at 59.50 min (peak 4), showed a protonated molecular ion at m/z 805.3270 that corresponds to a molecular composition of C₄₁H₄₉N₄O₁₃ (calcd. for C₄₁H₄₉N₄O₁₃, 805.3291) (Figure S2E, Supporting Information). The tandem MS spectrum revealed several product ions (Table 1) that denote the presence of a glucopyranosyl group at C-3² and a methyl ester group at C-8 (m/z 611.2509 [M+H-glucopyranosyl-MeOH]⁺, m/z 593.2388 [M+H-glucopyranosyl-MeOH-H₂O]⁺, and the ring A-glucopyranosyl, m/z 490.1968 [M+H-ring A-glucopyranosyl]⁺. The product ions fulfilled the filtering rules established for

Da) (Figure S2F, Supporting Information). The occurrence of key product ions observed at the tandem MS spectrum (Table 1) correlated with the characteristic fragmentation of **2**. Specifically, the loss of HOAc and the fragmentation of a glucopyranosyl moiety attached to ring A indicate a bicyclic structure (Table 1). Therefore, the MS²-based fragmentations for **4** were generated *in silico*, which were compared with the experimental product ions. The SmartFormula3D algorithm was applied to check their consistency according to the threshold values established for mass error and isotopic pattern. With this information, we tentatively identified **4** (YCC_787) as the oxidative derivative of **2**. In any case, it is important to highlight that, as it has previously been described,¹⁷ YCCs, unlike NCCs, do not show the fragmentation of the ring D during the MS/MS analysis. The characteristic double bond at C15-16 in YCCs probably hinders such fragmentation, but this situation is not possible in NCCs.

The catabolism of chlorophylls in pothos depicted in Scheme 1 through the structural characterization of the catabolite products has revealed significant issues. From the specific point of view of this evergreen tropical species, the catabolism is highly homogeneous as only type-I phyllobilins have been observed (featured by the formyl group at C-1), with an unsubstituted vinyl function at C-18, a methyl ester group at C-8², and hydroxylation at C-3² which is indeed esterified with a glucopyranosyl moiety. The structural differences appear at the C-12 propionic acid residue that is free in NCC_807/YCC_805, attached to the glucopyranosyl group in **2/4**, or esterified with a glucopyranosyl-3,4-dihydroxyphenethyl

reaction taking place at the chloroplast, yielding the FCC_645 (*Mc*-FCC-62, Figure S1, Supporting Information), a catabolite that is the substrate for the subsequent glucosylation either at C-3² or at C-12, a process that takes place at the cytosol. Although it has been suggested that the glucosylation process at the propionic acid chain could be performed by a different enzyme²⁸ this point should be experimentally demonstrated in the future, as well as the process that arranges the double esterification of the glucopyranosyl unit at C-3 and at the C-12 propionic acid residue.¹⁰

Epipremnum aureum (Linden & André) G.S.Bunting or pothos leaves constitute the fourth plant species, besides *Musa acuminata* (Colla), *Spatiphyllum wallisii* (Regel) and *Vitis vinifera* (L.) in which the exceptional *hm*FCCs have been detected. The new fluorescent catabolite (**1**) supports the principle that the *hm*FCCs identified so far share the following structural arrangements: they are type-I phyllobilins (possessing the formyl group at C-1), they are hydroxylated at C-3², and retain both the methyl ester function at the C-8² and the vinyl group at C-18, i.e., from all the possible arrangements observed in the final chlorophyll catabolites, only one structural modification (hydroxylation at C-3²) has been introduced in the original FCC structure. Consequently, the esterification at C-12 (*hm*FCCs) seems to hinder the action of the FCC-modifying enzymes such as the cytochrome P-450 CYP89A9, MES16 and the postulated enzyme responsible for the dihydroxylation at C-18. A hypothesis is that the striking arrangement at C-12 sterically hinders the enzymatic catalysis. Anyhow, it has been proposed

corresponds to the hydroxytyrosol-1-O-glucoside. Hydroxytyrosol, 2-(3,4-dihydroxyphenyl) ethanol is a phenol present in the fruits and leaves of the olive (*Olea europaea* L.), and is one of the main components of virgin olive oil.³⁴ Hydroxytyrosol is a renowned potent antioxidant,³⁵ with these major health benefits: cardioprotective, anti-inflammatory, with antimicrobial activity and antitumoral effects,³⁶ absorbed by humans.³⁷ In fact, the EFSA Panel on Dietetic Products, Nutrition and Allergies launched a scientific opinion on the substantiation of health claims related to hydroxytyrosol and derivatives in olives and the protection of LDL particles from oxidative damage.³⁸ It is the first time that a phenolic structure attached to a chlorophyll catabolite has been recognized in senescent tissues, an unprecedented arrangement in the characterization of natural products that opens up new perspectives in the study of chlorophyll catabolism, the aim of the process, and the strategies designed in the metabolic engineering of the plant during the senescence period. Hence, phenols in senescent tissues are related to the plant requirements of interaction with the environment, their reproductive strategy, and defense mechanisms toward stress.³⁹ In a senescent tissue where the photosynthetic apparatus is dismantled, the light radiation that is not further processed needs to be intercepted, or its deleterious effects (generation of reactive oxygen species, UV-damage) at least alleviated in some way. Here, the combination of two molecules, the FCC catabolite and the hydroxytyrosol glucoside, which may enhance the individual antioxidant activity of both molecular species, seems to gain singular relevance.

imported to the vacuole and transformed into NCCs, avoiding the oxidation of FCCs and consequently impairing the formation of YCCs from fluorescent phyllobilins. When senescent leaves of *S. wallisii* are left at room temperature and extracted with MeOH, two intermediates in the oxidation from NCCs to YCCs (the 15-hydroxy--NCC and the 15-hydroxymethyl-NCC) have been described.³² The authors suggest an “oxidative activity” as the plausible mechanism for the oxidation at C-15 in NCCs. However, the scenario has been complicated with the characterization of **1** formed in the cytosol. If a “biochemical” pathway is presupposed, then two different locations should be assumed for the same “oxidative” enzyme: cytosol and vacuole. Alternatively, an unspecific chemical oxidation should be hypothesized for the formation of YCCs. In this regard, the characterization, for the first time, of a bicyclic YCC (**4**) with an important inflexible bridge in its extraordinary rigid structure, points to the steric hindrance of an enzymatic reaction. In any case, the characterization of future new phyllobilins is necessary to complete the biochemical chlorophyll degradation pathway.

EXPERIMENTAL SECTION

Plant Material. Plants of *Epipremnum aureum* (Linden & André) G.S.Bunting (pothos) were obtained from a local garden center (Viveros Jardín del Rocío, Sevilla, Spain) and maintained indoor in daily bright indirect light without addition of any nutrient to the soils, and regular watering. Leaves that spontaneously entered in senescence (attached leaves) were left to get totally yellow, harvested, and processed for isolation of the chlorophyll catabolite

was obtained from a Milli-Q 50 system (Millipore Corp., Milford, MA, USA). Sodium formate (10 mM NaOH in 300 μ L of formic acid) was used for calibration.

Extraction of Chlorophyll Catabolites from Senescent Plant Tissues. The experimental procedures have been described previously¹⁷ and were made under diminished light. Briefly, fresh material (20 g of senescent leaves) was homogenized in liquid nitrogen and extracted into 10 volumes of ice-cold acetone during 30 min in a vortex at maximum speed at 4°C. The extract was centrifuged at 14000 $\times$ g for 5 min and the pellet was extracted again. Supernatants from both extractions were joined and concentrated in a rotary evaporator. SPE column (C-18, Bakerbond SPE, 500 mg/6 mL, J.T. Baker, Deventer, Holland) activated with two volumes of MeOH and two volumes of water was used to isolate the fraction of chlorophyll catabolites by applying to the SPE column the aqueous residue partially diluted with ice-cold MeOH (20%). Subsequently, the SPE with the sample was cleaned with four volumes of water for desalting, and the chlorophyll catabolites fraction was eluted with 1 mL of 20 mM K₃PO₄ pH 7.0/MeOH (1:9, v/v).^{41,42} The sample was stored at -20°C until analysis.

Liquid Chromatography/Electrospray Ionization/High-Resolution-Time-of-Flight Mass Spectrometry. Chromatographic separation was performed as described earlier⁴³ but with modifications detailed by Ríos et al.^{44,45} in a Dionex Ultimate3000RS U-HPLC (Thermo Fisher Scientific, Waltham, MA, USA). A split post-column of 0.4 mL/min was introduced directly on the mass spectrometer electrospray ion source. The HPLC/ESI-HRQqTOF operated

Germany). Subsequently, mass spectra were obtained focusing the acquisition of signals in two m/z ranges (900-1300 Da and 500-850 Da), so that the ionic abundance of the $[M+H]^+$ was maximized. Tandem-MS spectra were acquired in Auto-MS² mode (data-dependent acquisition) and were used for structural confirmation of compounds detected. Collision energy was estimated dynamically based on appropriate values for the mass and stepped across a $\pm 10\%$ magnitude range to ensure good quality fragmentation spectra.

Data Analysis. An *in house* mass database was created *ex professo* and comprises monoisotopic masses, elemental composition and retention time and characteristic fragment ions, for NCCs, DNCCs, YCCs and *hm*FCCs, if known,^{1,4,5,13-16,19,20,28} Additionally, elemental composition of new NCCs/DNCCs/YCCs/*hm*FCCs not described so far was included in the database.²⁵ Data evaluation was performed with Bruker Daltonics DataAnalysis 4.1. The software tools provided their elemental composition, which were compared with the built mass database. The MS scrutiny yielded the tentative identification of the chlorophyll catabolites. The experimental values of mass to charge ratio and isotopic pattern corresponding to those elemental compositions were compared with the theoretical data, obtaining the mass error and mSigma values, which should be below the tolerance limits (mass error <5 ppm and mSigma value <50). The consistency of the elemental composition and formulas of the experimental product ions was checked by applying the same procedure to the product ions and with the assistance of the SmartFormula3D algorithm.^{25,44,45} Indeed, the automated generation of

Structural arrangements of the six *hm*FCCs identified so far, and spectrometric data of compounds **1**, NCC_807, **2**, **3**, YCC_805 and **4**. Fragmentation scheme and *in silico* study of the alternative structural arrangements of **1**.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

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Table 1. MS Data for Peaks 1-6 Observed in the Analysis of Senescent Leaves of *Epipremnum aureum* by HPLC-HR-ESI-MS and Tandem HR-MS.

The MS² Fragmentations Numbers of Compound 1 Correspond with Those Indicated in Figures S3 and S4.

Compound	Peak number	t _R (min)	MS measurements				tandem-MS
			Error (ppm)	SigmaFit value	Molecular formula [M+H] ⁺	Calcd. m/z [M+H] ⁺	Product ions (m/z)
1	1	46.4	1.5	49.7	C ₅₅ H ₆₉ N ₄ O ₂₀	1105.4500	1. 1087.4386, [M+H-H ₂ O] ⁺ ; 2. 1073.4238, [M+H-MeOH] ⁺ ; 3. 982.3812, [M+H-ring D] ⁺ ; 4. 951.3844, [M+H-dihydroxyphenethyl] ⁺ ; 5. 943.3916, [M+H-glucopyranosyl] ⁺ ; 6. 915.3602, [M+H-C ₈ H ₁₃ O ₅] ⁺ ; 7. 789.3315, [M+H-glucopyranosyl-dihydroxyphenethyl] ⁺ ; 8. 753.3129, [M+H-glucopyranosyl-dihydroxyphenethyl-2×H ₂ O] ⁺ ;

							9. 636.2453, [M+H-glucopyranosyl-ring A-dihydroxyphenethyl] ⁺ ; 10. 341.1228 [M+H- C ₃₉ H ₄₆ N ₄ O ₁₁ -H ₂ O] ⁺
NCC_807	2	51.2	3.7	4.3	C ₄₁ H ₅₁ N ₄ O ₁₃	807.3447	775.3177, [M+H-MeOH] ⁺ ; 625.2622, [M+H-MeOH-glucopyranosyl] ⁺ ; 522.2228, [M+H-ring D-glucopyranosyl] ⁺
2	3	52.5	2.4	9.1	C ₄₁ H ₄₉ N ₄ O ₁₂	789.3342	757.3081, [M+H-MeOH] ⁺ ; 739.2957, [M+H-MeOH-H ₂ O] ⁺ ; 715.2975 [M+H-MeOH-HOAc] ⁺ ; 604.2292, [M+H-MeOH-H ₂ O-ring A] ⁺ ; 442.1764, [M+H-MeOH-ring A-glucopyranosyl] ⁺
3	4	56.9	3.7	18.4	C ₅₅ H ₆₇ N ₄ O ₂₀	1103.4343	941.3811, [M+H-glucopyranosyl] ⁺ ; 490.1973, [M+H-ring A-glucopyranosyl-glucopyranosyl-dihydroxyphenethyl] ⁺
YCC_805	5	59.5	-2.6	9.1	C ₄₁ H ₄₉ N ₄ O ₁₃	805.3291	611.2509 [M+H-glucopyranosyl-MeOH] ⁺ ; 593.2388 [M+H-glucopyranosyl-MeOH-H ₂ O] ⁺ ;

							490.1968 [M+H-ring A-glucopyranosyl] ⁺
4	6	61.3	-4.2	32	C ₄₁ H ₄₇ N ₄ O ₁₂	787.3185	755.2926, [M+H-MeOH] ⁺ ; 737.2823 [M+H-MeOH-H ₂ O] ⁺ ; 713.2820 [M+H-MeOH-HOAc] ⁺ ; 602.2128 [M+H-MeOH-H ₂ O-ring A] ⁺ ; 440.1605 [M+H-MeOH-ring A-glucopyranosyl] ⁺

TOC Graphic