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^{1}H and ^{13}C NMR spectroscopic structural elucidation of new decarboxylated betacyanins

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Abstract—Novel mono- and bi-decarboxylated betacyanins were generated by thermal decarboxylation of betacyanins of purple pitaya (*Hylocereus polyrhizus*) fruits and were identified by ^{1}H and ^{13}C NMR spectroscopy.

The betacyanin pigments have recently gained attention, being potential but thermolabile food colorants[1,2] and chemopreventive agents.[3] Following the need for identification, characterization and development of new and safe cancer chemopreventive agents, examination of vegetables, fruits, natural food colorants and herbal remedies for their cancer chemopreventive properties is currently being performed.[3]

Recent LC–MS/MS studies suggested the presence of decarboxylated betacyanins,[4–7] generated during heating of betacyanin preparations of *Beta vulgaris* L. roots and *Hylocereus polyrhizus* fruits in various solvents (alcohols and water). Moreover, 2-decarboxy-betanin and 2-decarboxy-phyllocactin were investigated in recent biosynthetic studies.[8] Whereas the structure of some 2-decarboxy-betacyanins (2-decarboxy-betanidin, -betanin and -phyllocactin) endogenously present in *Carpobrotus acinaciformis* and in studied hairy root cultures of *B. vulgaris* L. were proved by ^{1}H NMR,[8,9] no NMR structure elucidation was performed for the decarboxylated pigments in the preparations. Furthermore, there was one ^{13}C NMR study, but only conducted on non-decarboxylated betacyanins,[10] therefore, even if the presence of some of these compounds in betacyanin preparations was recently suggested,[4,5] they were not fully identified nor isolated. In addition, their potency for anti-cancer activity was not evaluated. The structures of these compounds had to be determined for further betacyanin decarboxylation mechanistic elucidations. Taking this into account, extensive NMR studies on decarboxylated betacyanins were necessary for unambiguous confirmation of the structures.

In this contribution we report the results of ^{1}H and ^{13}C NMR structure elucidation of 2-monodecarboxy-betacyanins, 17-monodecarboxy-betacyanins and 2,17-bidecarboxy-betacyanins (Fig. 1), generated by decarboxylation of 10–20 mg of chromatographically isolated betacyanins (betanin, phyllocactin and hylocerenin) from *H. polyrhizus* fruits.

Recent reports on betacyanin decarboxylation of *B. vulgaris* L. and *H. polyrhizus* pigments suggested their fast degradation in ethanolic solutions leading to single and double decarboxylation.[7] Moreover, different initial products of monodecarboxylation in ethanolic and aqueous solutions of betacyanins were identified,[6,7] indicating different decarboxylation mechanisms in ethanol and in water. Therefore, in this study both solvents were used for the selective decarboxylation of betacyanins derived from *H. polyrhizus* extracts. Decarboxylation was performed in betacyanin 300 mL aqueous or 50 mL ethanolic solutions, acidified with 500 μL of glacial acetic acid, by heating them for 30 min at 85 °C (aqueous solutions) and for 10 min at 75 °C (ethanolic solutions).

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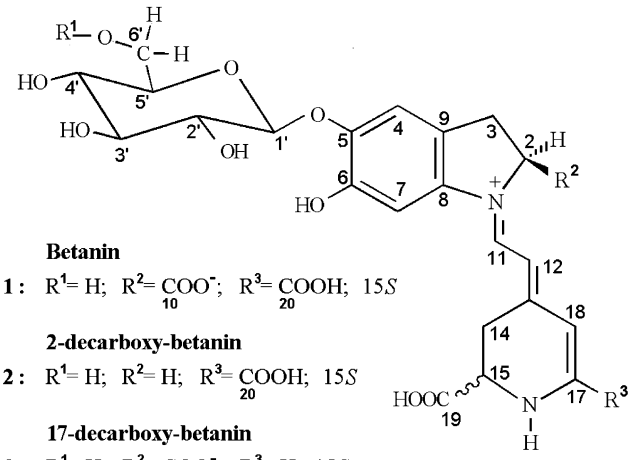

Betanin

1: R^1= H; R^2= COO^-_{10}; R^3= $COOH_{20}$; 15*S*

2-decarboxy-betanin

2: R^1= H; R^2= H; R^3= $COOH_{20}$; 15*S*

17-decarboxy-betanin

3: R^1= H; R^2= COO^-_{10}; R^3= H; 15*S*

2,17-bidecarboxy-betanin

4: R^1= H; R^2= H; R^3= H; 15*S*

2-decarboxy-phyllocactin

5: R^1= $CO_{1''}CH_2{}_{2''}COOH_{3''}$; R^2= H; R^3= $COOH_{20}$; 15*S*

2-decarboxy-hylocerenin

6: R^1= $CO_{1''}CH_2{}_{2''}C(OH)(CH_3)_{4''}CH_2{}_{5''}COOH_{6''}$; R^2= H; R^3= $COOH_{20}$; 15*S*

17-decarboxy-phyllocactin

7: R^1= $CO_{1''}CH_2{}_{2''}COOH_{3''}$; R^2= COO^-_{10}; R^3= H; 15*S*

2,17-bidecarboxy-phyllocactin

8: R^1= $CO_{1''}CH_2{}_{2''}COOH_{3''}$; R^2= H; R^3= H; 15*S*

2,17-bidecarboxy-hylocerenin

9: R^1= $CO_{1''}CH_2{}_{2''}C(OH)(CH_3)_{4''}CH_2{}_{5''}COOH_{6''}$; R^2= H; R^3= H; 15*S*

Figure 1. Chemical structures of betanin as well as the 2-decarboxy-, 17-decarboxy- and 2,17-bidecarboxy-betacyanins studied.

For all the investigated compounds (**1**–**9**), the ^{1}H NMR, 1D TOCSY, gCOSY and NOESY spectra in D_2O[11–19] allowed us to distinguish and assign various coupled spin systems ^{13}C chemical shifts for all carbons directly bound to protons were unambiguously assigned by gHSQC correlations. The ^{1}H–^{13}C long-range correlations (gHMBC) allowed us to establish the chemical shifts of the quaternary carbons.

The doublets (or broad singlets) for the H-11 and H-12 protons were readily distinguishable by their low- and high-field shifts, respectively. H-4 and H-7 appeared as singlets and were unambiguously assigned by NOESY correlation between H-4 and H-3a/b. A small chemical shift difference in H-4 and H-7 (0.08–0.15 ppm), as well as the NOE between H-4 and H-1′ indicated the presence of a hydroxyl group at C-6 of the betanidin unit. A three-bond vicinal coupling constant $^3J_{\text{H-1}',\text{H-2}'} \sim 7$ Hz, indicated the β-linkage between the aglycone and glucopyranosyl moiety.

As previously shown,[10] the observed NOESY correlations between H-7 and H-11 as well as H-2 and H-12 confirmed the *s-trans* conformation of the dienyl system (N-1, C-11, C-12, C-13). Additionally, the NOE's between the H-12 and H-18, as well as the H-11 and H-14 protons were observed, which together with the long-range correlation between H-12 and the C-13 carbon at ~117.8 ppm (for **1**,[11] **2**[12] and **5**[15]) proved the *E*-configuration of the C-12/C-13 double bond.

The ^{1}H spin system of the glucopyranosyl moiety (H-1′–H-6′) was established by 1D TOCSY and gCOSY as well as from the glucopyranosyl ^{13}C chemical shifts, which were assigned by gHSQC. In the case of the acylated compounds the observed downfield shifts for the glucopyranosyl proton signals, H-6′a and H-6′b in the ^{1}H NMR spectra and C-6′ in the gHSQC, in comparison to the betanin derived structures, were clear evidence that the acyl system was bound to C-6′ of the glucose moiety.

The signals resulting from the presence of the malonyl moiety in **5**,[15] **7**[17] and **8**[18] were not visible in the gHSQC and gHMBC spectra obtained for the samples dissolved in D_2O, due to the fast H/D exchange of the H-2″ a/b protons, which was also noticed recently for phyllocactin.[10] Therefore, these samples were also analyzed in a mixture of H_2O and D_2O (90/10, v/v),[10] which revealed the malonyl H-2″ a/b protons and the C″-2 carbon in each case, however, the lack of sufficient quantities of **7** and **8** prevented the acquisition of gHMBC data for C″-1 and C″-3. The presence of the malonyl moiety in **5**, **7** and **8** as well as the 3-hydroxy-3-methyl-glutaryl unit in **6**[16] and **9**[19] has been recently suggested from the LC–MS/MS data.[7] The signals of the 3-hydroxy-3-methyl-glutaryl moiety in **6** and **9** were readily distinguished by ^{1}H NMR, gHSQC and gHMBC.

The mono- or bidecarboxylation at C-2 and/or C-17 carbons of the starting betanin, phyllocactin and hylocerenin substrates (Fig. 1) resulted primarily in the formation of the individual ^{1}H-spin systems: H-2a/b and H-3a/b, and/or H-17 and H-18, respectively. For the 2-decarboxy- and 2,17-bidecarboxy-betacyanins a broad triplet upfield signal at $\delta \approx 4.10$ ppm for the two H-2a/b protons appeared in comparison to the betanin-derived H-2 proton double doublet at $\delta = 4.86$ ppm. Additionally, the ~0.5 ppm upfield shifts of the H-3a/b proton signal and the upfield changes of the ^{13}C chemical shifts derived from gHSQC for the C-2 and C-3 carbons confirmed the decarboxylation at C-2.

For 17-decarboxy- and 2,17-bidecarboxy-betacyanins a new H-17 proton doublet signal at $\delta \sim 7.50$ ppm and an additional signal for the C-17 carbon at $\delta =$ 155.3 ppm were established by gHSQC and gHMBC, confirming the lack of the carboxyl group at C-17. The formation of the H-17 and H-18 ^{1}H-spin system was further observed by the upfield change of the H-18 proton broad signal to δ ~5.76 ppm. For all the studied compounds a new signal for C-18 derived from gHSQC and gHMBC spectra was assigned at $\delta \sim 105.2$ ppm for the first time, however, no significant changes in the value were observed in transition from betanin to the 17- and 2,17-decarboxylated structures.

New signals for the C-10, C-19 and C-20 carbons were assigned by gHMBC only in a few cases.

Attempts to differentiate the configurational stereoisomers at C-15 (Fig. 1), which is a difficult task,[2] were not successful for any of the studied pairs of pigments, therefore the data of only one compound per pair was presented. Because the elution order of 2,17-bidecarboxy-betacyanin C-15 stereoisomers in reversed phase HPLC could not be established in the recent study,[7] their C-15 configuration still remains to be solved.

The ^{1}H and ^{13}C NMR data from this study together with the recently reported other spectroscopic data (UV–vis and LC–ESI-MS/MS),[5,6] allowed the unambiguous structure elucidation of all the mono- and bidecarboxylated betacyanins generated from heating *B. vulgaris* L. and *H. polyrhizus* preparations. For the first time the ^{13}C NMR spectra of decarboxylated betacyanins were obtained and compared to the data of the recent structural study[10] on non-decarboxylated betacyanins. Whereas the ^{1}H NMR assignment for 2-decarboxy-betanin and 2-decarboxy-phyllocactin have already been performed,[8] in this study we received the first ^{1}H NMR spectra of 2-decarboxy-hylocerenin together with all the studied 17-decarboxy and 2,17-bidecarboxy-betacyanins.

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11. NMR data for **1** (betanin[10]): ^{1}H NMR (600 MHz, D_2O): δ 3.11 (1H, dd, $J = 3.2$, 16.4 Hz, H-3B), 3.16 (1H, br m, H-14B), 3.26 (1H, br m, H-14A), 3.42 (1H, overlap, H-4′), 3.52 (1H, overlap, H-5′), 3.53 (1H, overlap, H-3′), 3.55 (1H, overlap, H-2′), 3.58 (1H, dd, $J = 10.5$, 16.7 Hz, H-3A), 3.71 (1H, dd, $J = 5.3$, 12.2 Hz, H-6′B), 3.86 (1H, dd, $J = 1.8$, 12.5 Hz, H-6′A), 4.29 (1H, br t, $J = 7.3$ Hz, H-15), 4.86 (1H, dd, $J = 3.1$, 10.3 Hz, H-2), 4.99 (1H, d, $J = 7.1$ Hz, H-1′), 5.82 (1H, br d, $J = 12.2$ Hz, H-12), 6.21 (1H, br s, H-18), 7.01 (1H, s, H-7), 7.09 (1H, s, H-4), 8.19 (1H, br d, $J = 11.4$ Hz, H-11); ^{13}C NMR (150 MHz, D_2O): δ 27.5 (C-14), 33.8 (C-3), 54.3 (C-15), 60.7 (C-6′), 65.6 (C-2), 69.7 (C-4′), 73.2 (C-3′), 75.9 (C-2′), 76.8 (C-5′), 100.0 (C-7), 101.9 (C-1′), 105.2 (C-18), 106.2 (C-12), 114.0 (C-4), 117.7 (C-13), 124.5 (C-9), 138.3 (C-8), 143.9 (C-5), 144.0 (C-11), 146.6 (C-6), 177.1 (C-10).
12. NMR data for **2** (2-decarboxy-betanin): ^{1}H NMR (600 MHz, D_2O): δ 3.09 (1H, br m, H-14B), 3.16 (2H, br t, H-3A/B), 3.23 (1H, br m, H-14A), 3.41 (1H, overlap, H-4′), 3.53 (1H, overlap, H-5′), 3.53 (1H, overlap, H-3′), 3.55 (1H, overlap, H-2′), 3.71 (1H, br dd, $J = 5.2$, 12.3 Hz, H-6′B), 3.87 (1H, br d, $J = 12.3$ Hz, H-6′A), 4.11 (2H, br t, $J = 5.6$ Hz, H-2A/B), 4.23 (1H, br t, $J = 7.4$ Hz, H-15), 4.98 (1H, d, $J = 6.7$ Hz, H-1′), 6.02 (1H, br d, $J = 12.4$ Hz, H-12), 6.17 (1H, br s, H-18), 6.98 (1H, s, H-7), 7.08 (1H, s, H-4), 8.17 (1H, br d, $J = 11.0$ Hz, H-11); ^{13}C NMR (150 MHz, D_2O): δ 26.6 (C-3), 27.0 (C-14), 50.1 (C-2), 53.8 (C-15), 60.4 (C-6′), 69.3 (C-4′), 72.8 (C-3′), 75.2 (C-2′), 76.0 (C-5′), 99.7 (C-7), 101.3 (C-1′), 104.1 (C-18), 106.3 (C-12), 113.7 (C-4), 117.4 (C-13), 124.1 (C-9), 136.9 (C-8), 143.3 (C-5), 144.0 (C-11), 145.8 (C-6).
13. NMR data for **3** (17-decarboxy-betanin): ^{1}H NMR (600 MHz, D_2O): δ 3.11 (1H, dd, $J = 3.4$, 16.6 Hz, H-3B), 3.15 (2H, br m, H-14A/B), 3.40 (1H, overlap, H-4′), 3.46 (1H, overlap, H-3′), 3.49 (1H, overlap, H-5′), 3.49 (1H, overlap, H-2′), 3.50 (1H, dd, $J = 10.5$, 16.6 Hz, H-3A), 3.64 (1H, dd, $J = 5.3$, 12.4 Hz, H-6′B), 3.79 (1H, dd, $J = 1.6$, 12.4 Hz, H-6′A), 4.18 (1H, t, $J = 7.9$ Hz, H-15), 4.72 (1H, dd, $J = 3.4$, 10.5 Hz, H-2), 4.90 (1H, d, $J = 7.0$ Hz, H-1′), 5.57 (1H, br s, H-12), 5.76 (1H, br s, H-18), 6.88 (1H, s, H-7), 7.00 (1H, s, H-4), 7.52 (1H, d, $J = 5.7$ Hz, H-17), 8.03 (1H, br s, H-11); ^{13}C NMR (150 MHz, D_2O): δ 27.0 (C-14), 33.0 (C-3), 53.7 (C-15), 60.3 (C-6′), 64.8 (C-2), 69.3 (C-4′), 72.7 (C-3′), 75.9 (C-2′), 76.0 (C-5′), 99.2 (C-7), 101.5 (C-1′), 103.8 (C-12), 105.3 (C-18), 113.8 (C-4), 123.5 (C-9), 137.9 (C-8), 142.8 (C-11), 143.0 (C-5), 146.1 (C-6), 155.3 (C-17), 176.0 (C-19), 176.7 (C-10).
14. NMR data for **4** (2,17-bidecarboxy-betanin): ^{1}H NMR (600 MHz, D_2O): δ 3.15 (2H, br t, H-3A/B), 3.20 (2H, br m, H-14A/B), 3.41 (1H, overlap, H-4′), 3.52 (1H, overlap, H-5′), 3.53 (1H, overlap, H-3′), 3.55 (1H, overlap, H-2′), 3.71 (1H, dd, $J = 5.2$, 12.5 Hz, H-6′B), 3.86 (1H, dd, $J = 2.2$, 12.5 Hz, H-6′A), 4.06 (2H, br t, $J = 7.5$ Hz, H-2A/B), 4.20 (1H, t, $J = 8.3$ Hz, H-15), 4.97 (1H, d, $J = 7.9$ Hz, H-1′), 5.78 (1H, br s, H-18), 5.84 (1H, br s, H-12), 6.94 (1H, s, H-7), 7.08 (1H, s, H-4), 7.52 (1H, d, $J = 5.5$ Hz, H-17), 8.10 (1H, br s, H-11); ^{13}C NMR (150 MHz, D_2O): δ 27.1 (C-14), 26.7 (C-3), 49.7 (C-2), 53.8 (C-15), 60.4 (C-6′), 69.2 (C-4′), 72.8 (C-5′), 75.6 (C-3′), 76.0 (C-2′), 99.4 (C-7), 101.4 (C-1′), 104.4 (C-18), 109.1 (C-12), 114.0 (C-4), 125.8 (C-9), 138.5 (C-11), 137.5 (C-8), 143.6 (C-5), 145.9 (C-6), 154.4 (C-17).
15. NMR data for **5** (2-decarboxy-phyllocactin): ^{1}H NMR (600 MHz, D_2O): δ 3.10 (1H, br m, H-14B), 3.13 (2H, br t, H-3A/B), 3.21 (1H, br m, H-14A), 3.25[†] (2H, d, H-2″A/B), 3.53 (1H, overlap, H-4′), 3.55 (1H, overlap, H-2′), 3.56 (1H, overlap, H-3′), 3.75 (1H, m, H-5′), 4.07 (2H, br t, $J = 5.6$ Hz, H-2A/B), 4.24 (1H, br t, $J = 7.2$ Hz, H-15), 4.35 (1H, dd, $J = 4.0$, 12.4 Hz, H-6′B), 4.40 (1H, dd,

[†] The NMR signals were observed only in H_2O/D_2O solution (9/1, v/v) due to fast H/D exchange on the malonyl side chain.

$J = 12.4$ Hz, H-6′A), 4.97 (1H, d, $J = 6.6$ Hz, H-1′), 5.98 (1H, br d, $J = 11.0$ Hz, H-12), 6.17 (1H, s, H-18), 6.93 (1H, s, H-7), 7.03 (1H, s, H-4), 8.10 (1H, br d, $J = 10.9$ Hz, H-11); ^{13}C NMR (150 MHz, D_2O): δ 26.9 (C-3), 27.0 (C-14), 44.1† (C-2″), 50.0 (C-2), 53.9 (C-15), 63.5 (C-6′), 69.3 (C-4′), 72.8 (C-3′), 73.7 (C-5′), 75.3 (C-2′), 99.6 (C-7), 101.0 (C-1′), 104.1 (C-12), 106.7 (C-18), 113.3 (C-4), 118.1 (C-13), 126.4 (C-9), 137.1 (C-8), 143.7 (C-11), 143.8 (C-5), 145.6 (C-6), 170.9† (C-1″), 172.6† (C-3″).

16. NMR data for **6** (2-decarboxy-hylocerenin): ^{1}H NMR (600 MHz, D_2O): δ 1.22 (3H, s, H-3″), 2.41 (1H, d, $J = 15.1$ Hz, 5″A), 2.46 (1H, d, $J = 15.1$ Hz, 5″B) 3.12 (1H, br m, H-14B), 3.16 (2H, br t, H-3A/B), 3.26 (1H, br m, H-14A), 2.60 (1H, d, $J = 14.5$ Hz, H-2″A), 2.64 (1H, d, $J = 14.5$ Hz, H-2″B), 3.48 (1H, overlap, H-4′), 3.57 (1H, overlap, H-2′), 3.56 (1H, overlap, H-3′), 3.74 (1H, m, H-5′), 4.11 (2H, br t, $J = 7.2$ Hz, H-2A/B), 4.24 (1H, dd, $J = 11.5$ Hz, H-6′B), 4.25 (1H, br t, $J = 7.1$ Hz, H-15), 4.46 (1H, dd, $J = 11.5$ Hz, H-6′A), 5.10 (1H, d, $J = 6.6$ Hz, H-1′), 6.02 (1H, br d, $J = 8.8$ Hz, H-12), 6.18 (1H, s, H-18), 6.97 (1H, s, H-7), 7.05 (1H, s, H-4), 8.16 (1H, br d, $J = 8.8$ Hz, H-11); ^{13}C NMR (150 MHz, D_2O): δ 26.3 (C-4″), 26.8 (C-3), 27.1 (C-14), 45.5 (C-2″), 45.8 (C-5″), 50.0 (C-2), 53.9 (C-15), 63.3 (C-6′), 69.5 (C-4′), 69.9 (C-3″), 72.9 (C-3′), 73.6 (C-5′), 75.0 (C-2′), 99.6 (C-7), 101.0 (C-1′), 104.1 (C-18), 106.5 (C-12), 113.3 (C-4), 126.2 (C-9), 137.4 (C-8), 143.7 (C-11), 143.8 (C-5), 146.7 (C-6), 172.6 (C-6″), 177.1 (C-1″).
17. NMR data for **7** (17-decarboxy-phyllocactin): ^{1}H NMR (600 MHz, D_2O): δ 3.08 (1H, dd, $J = 3.5$, 16.2 Hz, H-3B), 3.21 (2H, br d, $J = 7.8$ Hz, H-14A/B), 3.22† (2H, d, H-2″A/B), 3.56 (1H, overlap, H-3′), 3.56 (1H, overlap, H-4′), 3.57 (1H, overlap, H-5′), 3.58 (1H, dd, $J = 10.4$, 16.2 Hz, H-3A), 3.73 (1H, m, H-2′), 4.24 (1H, t, $J = 8.2$ Hz, H-15), 4.35 (1H, dd, $J = 4.0$, 12.4 Hz, H-6′B), 4.40 (1H, dd, $J = 12.4$ Hz, H-6′A), 4.78 (1H, dd, $J = 3.5$, 10.3 Hz, H-2), 4.96 (1H, d, $J = 7.2$ Hz, H-1′), 5.63 (1H, br s, H-12), 5.84 (1H, br s, H-18), 6.95 (1H, s, H-7), 7.05 (1H, s, H-4), 7.59 (1H, d, $J = 5.6$ Hz, H-17), 8.10 (1H, br s, H-11); ^{13}C NMR (150 MHz, D_2O): δ 27.2 (C-14), 33.2 (C-3), 44.0† (C-2″), 53.9 (C-15), 63.2 (C-6′), 64.9 (C-2), 68.9 (C-4′), 73.1 (C-3′), 73.7 (C-5′), 74.9 (C-2′), 99.4 (C-7), 101.9 (C-1′), 104.0 (C-12), 105.5 (C-18), 114.5 (C-4), 123.3 (C-9), 138.3 (C-8), 142.6 (C-5), 142.9 (C-11), 146.1 (C-6), 155.3 (C-17).
18. NMR data for **8** (2,17-bidecarboxy-phyllocactin): ^{1}H NMR (600 MHz, D_2O): δ 3.14 (2H, br t, H-3A/B), 3.16 (2H, br m, H-14A/B), 3.29† (2H, d, H-2″A/B), 3.52 (1H, overlap, H-4′), 3.55 (1H, overlap, H-3′), 3.57 (1H, overlap, H-2′), 3.77 (1H, m, H-5′), 4.02 (2H, br t, $J = 7.1$ Hz, H-2A/B), 4.19 (1H, t, $J = 8.3$ Hz, H-15), 4.32 (1H, dd, $J = 5.3$, 12.3 Hz, H-6′B), 4.46 (1H, dd, $J = 12.1$ Hz, H-6′A), 4.94 (1H, d, $J = 5.8$ Hz, H-1′), 5.76 (1H, br s, H-18), 5.80 (1H, br s, H-12), 6.89 (1H, s, H-7), 7.03 (1H, s, H-4), 7.51 (1H, br s, H-17), 8.05 (1H, br s, H-11); ^{13}C NMR (150 MHz, D_2O): δ 26.8 (C-3), 27.1 (C-14), 44.6† (C-2″), 49.7 (C-2), 53.9 (C-15), 63.4 (C-6′), 69.2 (C-4′), 73.0 (C-3′), 73.7 (C-5′), 74.6 (C-2′), 99.3 (C-7), 101.1 (C-1′), 104.2 (C-12), 104.2 (C-18), 113.4 (C-4), 125.6 (C-9), 137.4 (C-8), 143.1 (C-5), 143.3 (C-11), 145.7 (C-6), 154.2 (C-17).
19. NMR data for **9** (2,17-bidecarboxy-hylocerenin): ^{1}H NMR (600 MHz, D_2O): δ 1.19 (3H, s, H-3″), 2.42 (1H, d, $J = 15.1$ Hz, 5″A), 2.45 (1H, d, $J = 15.0$ Hz, 5″B), 2.61 (1H, d, $J = 14.5$ Hz, H-2″A), 2.63 (1H, d, $J = 14.6$ Hz, H-2″B), 3.14 (1H, br m, H-14B), 3.15 (2H, br t, $J = 7.6$ Hz, H-3A/B), 3.21 (1H, br m, H-14A), 3.49 (1H, overlap, H-4′), 3.55 (1H, overlap, H-3′), 3.57 (1H, overlap, H-2′), 3.74 (1H, m, H-5′), 4.06 (2H, br t, $J = 6.9$ Hz, H-2A/B), 4.20 (1H, t, $J = 8.5$ Hz, H-15), 4.26 (1H, dd, $J = 6.0$, 12.0 Hz, H-6′B), 4.46 (1H, dd, $J = 12.0$ Hz, H-6′A), 5.00 (1H, d, $J = 7.4$ Hz, H-1′), 5.78 (1H, br s, H-18), 5.83 (1H, br s, H-12), 6.94 (1H, s, H-7), 7.05 (1H, s, H-4), 7.51 (1H, br d, $J = 5.6$ Hz, H-17), 8.11 (1H, br s, H-11); ^{13}C NMR (150 MHz, D_2O): δ 26.2 (C-4″), 26.6 (C-3), 27.1 (C-14), 45.7 (C-2″), 47.3 (C-5″), 49.9 (C-2), 53.8 (C-15), 63.2 (C-6′), 69.7 (C-3″), 69.9 (C-4′), 72.5 (C-3′), 73.8 (C-5′), 75.1 (C-2′), 99.5 (C-7), 101.3 (C-1′), 104.3 (C-12), 104.7 (C-18), 114.1 (C-4), 125.8 (C-9), 137.9 (C-8), 139.2 (C-11), 143.0 (C-5), 146.3 (C-6), 154.4 (C-17), 177.1 (C-1″), 172.8 (C-6″).