7-*O*-Methylcyanidin 3-*O*-β-D-Galactopyranoside, a Novel Anthocyanin from Mango (*Mangifera indica* L. cv. 'Tommy Atkins') Peels

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Mango (*Mangifera indica* L.) is one of the most important tropical fruits and its pulp is known to be a rich source of carotenoids. Investigations on the phenolic composition of the peels of a red-colored cultivar (cv. 'Tommy Atkins') revealed the presence of a new anthocyanin, 7-*O*-methylcyanidin 3-*O*-β-D-galactopyranoside. Its structure was elucidated by extensive 1D and 2D NMR studies, MS, and chemical transformation.

Key words: *Mangifera indica* (L.) cv. 'Tommy Atkins', Anthocyanin, 7-*O*-Methylcyanidin 3-*O*-β-D-Galactopyranoside, ESI(+)-MS^2, NMR

Introduction

With a global production exceeding 25 million tons in 2003, mangos (*Mangifera indica* L., Anacardiaceae) represent, beside bananas, one of the most important tropical fruits [1]. Mangos are a rich source of carotenoids, with β-carotene accounting for more than half of the total carotenoid content in most cultivars. In tropical countries mangos substantially contribute to the β-carotene supply. While the mango carotenoids have been investigated extensively [2], studies on the phenolic compounds, in particular on the anthocyanins in the peels of red-colored cultivars, are scarce. Recent investigations have shown that the peels of mango fruit contain a number of flavonol and xanthone glycosides as well as gallotannins and benzophenone derivatives [3, 4]. During our studies on the profile of mango peel phenolics, two anthocyanins were detected in red-colored cultivars by HPLC-DAD [5]. The minor anthocyanin could be identified as cyanidin 3-*O*-galactoside by comparison of the HPLC retention time and LC-MS spectrum with reference material. The molecular mass of 463 Da of the predominant anthocyanin suggested the presence of peonidin 3-*O*-galactoside, the only mango peel anthocyanin described in literature so far [6]. However, HPLC experiments with reference compounds could neither confirm the presence of peonidin 3-*O*-galactoside nor of peonidin 3-*O*-glucoside. Therefore, the objective of the present study was the isolation and structure elucidation of the unknown anthocyanin. It could be unambiguously identified as 7-*O*-methylcyanidin 3-*O*-β-D-galactopyranoside (**1**). To the best of our knowledge, this is the first report on 7-*O*-methylcyanidin 3-*O*-β-D-galactopyranoside. Furthermore, the anthocyanidin 7-*O*-methylcyanidin has been characterized for the first time using modern analytical methods such as ESI(+)-MS^2, HRFABMS and NMR.

Result and Discussion

Mango peels (cv. 'Tommy Atkins') were lyophilized, ground and extracted with aqueous acetone. The extract was purified using a C_{18}-Sep-Pak cartridge. Final clean-up of the anthocyanin was achieved by semipreparative reversed-phase HPLC. 7-*O*-Methylcyanidin 3-*O*-β-D-galactopyranoside (**1**) was obtained as bluish-red amorphous powder, $[\alpha]_D^{25}$ – 237 (*c* 0.11, 0.1% TFA). The UV/vis spectrum in MeOH, acidified with 0.1% HCl, showed a maximum at 523 nm typical of anthocyanins. The HRFABMS measurements exhibited a $[M]^+$ ion of *m/z* 463.1203, corresponding to the molecular formula $C_{22}H_{23}O_{11}$. Collision-induced fragmentation during the ESI(+)-MS^2 of the anthocyanin yielded the predominant fragment with *m/z* 301. The loss of 162 amu indicated

the presence of a hexose moiety. The observation of an anomeric proton at $\delta = 5.00$ (d, $J = 7.6$ Hz, 1"-H) together with six protons in the region $\delta = 3.70-3.91$ confirmed the presence of a sugar moiety in **1**. The connectivities of the seven sugar protons were determined by COSY and 1D-TOCSY, and their directly bonded carbons were assigned by GHSQC. The observed coupling constant of $J = 7.6$ Hz for the anomeric proton suggested a β-linkage of the sugar moiety to the aglycone. A vicinal coupling constant of $J = 9.6$ Hz between 2"-H ($\delta = 3.77$) and 3"-H ($\delta = 3.70$), $J = 3.9$ Hz between 3"-H and 4"-H ($\delta = 3.91$, bd) along with NOE's between 1"-H and 3"-H as well as 1"-H and 5"-H established a galactopyranosyl moiety with $^4C_1$ conformation in the case of a D configurated pyranose. Further analysis of the ^{1}H NMR, the COSY spectrum and 1D TOCSY spectra revealed an aromatic ABM splitting system consisting of the protons at $\delta = 6.91$ (bs, 2'-H), $\delta = 6.20$ (d, $J = 7.8$ Hz, 5'-H) and $\delta = 7.22$ (bd, $J = 7.8$ Hz, 6'-H), a set of two *meta*-coupled protons at $\delta = 6.13$ (bs, 6-H) and $\delta = 6.19$ (bs, 8-H), a lowfield-shifted proton at $\delta = 8.16$ (bs, 4-H), and a methoxy group at $\delta = 3.63$ (s). The ^{13}C chemical shifts of their corresponding carbons C-2' ($\delta = 118.1$), C-5' ($\delta = 118.3$), C-6' ($\delta = 128.7$), C-6 ($\delta = 103.4$), C-8 ($\delta = 94.0$), C-4 ($\delta = 134.5$) were assigned by the GHSQC experiment. By combining all the ^{1}H-^{13}C long range correlations (HMBC) of the aromatic protons and the remaining nine quaternary aromatic carbons, the anthocyanidin structure for the aglycone was established. HMBC-correlations between the methoxy group and the quaternary carbon at $\delta = 170.6$ (C-7) along with ^{1}H-^{13}C long range correlations between both 6-H and C-7 and 8-H and C-7 indicated that the methoxy group is attached to the A-ring. ROESY crosspeaks between the methoxy group and 6-H as well as 8-H unambiguously established the C-7 position of the methoxy group. Furthermore, the HMBC correlation between the anomeric proton 1"-H and C-4 at $\delta = 134.5$ proved the glycosidic linkage of the galactopyranosyl moiety to C-4 of the aglycone. Acid hydrolysis (2 N HCl) of **1** yielded the aglycone, 7-*O*-methylcyanidin (**2**), and the sugar moiety, which was identified as D-galactose by ^{1}H NMR and optical rotation ($[\alpha]_D^{25} = +85$) in comparison with an authentic sample. The structure of 7-*O*-methylcyanidin (**2**) was confirmed by MS and extensive 1D and 2D NMR including GCOSY, ROESY, GHSQC and GHMQC. In the ROESY spectrum of **2** crosspeaks between the two aromatic B-ring

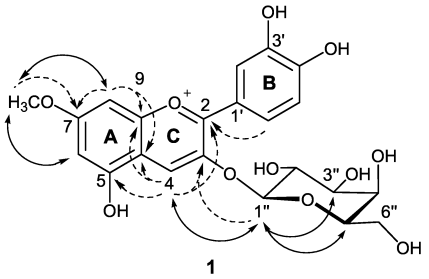

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Fig. 1. Structure of 7-*O*-methylcyanidin 3-*O*-β-D-galactopyranoside (**1**). Arrows indicate important HMBC [$\dashrightarrow$] and ROESY [$\longleftrightarrow$] correlations.

protons 2'-H/6'-H and the aromatic A-ring proton at $\delta = 7.15$ ($\delta_C = 92.3$) proved its 8-position and thus the 6-position of its *meta*-coupled partner proton at $\delta = 6.68$ ($\delta_C = 102.9$). Despite the fact that NMR data of the aglycone **2** are not available in the literature, the observed NMR data (see experimental) are in good agreement with the NMR data of similar compounds like cyanidin and cyanidin glycosides [7–9].

In a previous study, an anthocyanin from the flowers of *Bombax malabaricum* DC (Bombacaceae) was isolated and its structure assigned to 7-*O*-methylcyanidin 3-*O*-glucoside by paper-chromatography, IR- and UV/vis spectroscopy, and chemical reactions [10]. Hitherto, 7-*O*-methylcyanidin is not considered in reviews concerning anthocyanidins [11, 12], and, to the best of our knowledge, the present work marks the first report on 7-*O*-methylcyanidin using modern analytical methods such as ESI(+)-MS and NMR.

Experimental Section

General experimental procedures

Optical rotation was measured on a Perkin-Elmer model 341 polarimeter with 0.1% aqueous TFA as the solvent. UV spectra were recorded in MeOH, acidified with 0.1% HCl, on a Perkin-Elmer Lambda 20 instrument. IR spectra were obtained using a Perkin-Elmer FT-IR spectrometer. ESI(+)-MS analyses were performed using a Bruker Esquire 3000+ ion trap mass spectrometer fitted with an ESI source. ^{1}H NMR data were obtained on a Varian Unity Inova 500 MHz spectrometer. The ^{1}H and ^{13}C chemical shifts were referenced to solvent signals at $\delta_{H/C}$ 3.32/49.0 (methanol-d_4 containing 5% TFA) and $\delta_{H/C}$ 4.70 $_{(HDO)}$/164.5 $_{(TFA)}$ (D_2O containing 1% TFA) relative to TMS. All 1D (^{1}H, DPFGNOE, TOCSY) and 2D (GCOSY, ROESY, GHSQC, GHMQC, G = "gradient enhanced") NMR measurements were performed using standard Varian pulse sequences. ^{1}H-^{13}C correlation spectra were recorded by GHSQC ($J_{C-H} = 140$ Hz) for the determination of proton-bearing carbons and

GHMQC ($^nJ_{C-H}$ optimized for 8 Hz) for multibond correlations (HMBC). ROESY experiments were carried out with 0.5 s mixing time. ^{13}C NMR data were obtained on a Varian Unity Inova 300 MHz spectrometer.

Plant material

Mature Peruvian mango fruits (cv. 'Tommy Atkins') obtained from the local market were washed and the peels were removed with a stainless steel knife. After the separation of residual pulp from the peel with a razor blade, the samples were immediately lyophilized and finely ground.

Extraction and isolation

Lyophilized peels (125 g) were extracted with aqueous acetone (80 %, v/v; 1% ascorbic acid). The organic solvent was evaporated from the extract *in vacuo* at 30 °C. Aliquots of 30 ml of the aqueous extract were applied on a 10 g C_{18}-Sep-Pak cartridge. Non-anthocyanin substances were subsequently eluted with 0.01% HCl and EtOAc. The anthocyanins were eluted with acidified MeOH (0.01% hydrochloric acid) and the eluate was evaporated *in vacuo* to dryness. Using a Bischoff Nitrile column (250 × 15 mm i.d., particle size 5 μm), the anthocyanin was purified by semipreparative HPLC. The mobile phase consisted of 87% (v/v) water, 0.1% TFA, 3% acetonitrile (eluent A) and 50% water, 0.1% TFA, 50% acetonitrile (eluent B). The gradient program was as follows: 0% B (5 min), 0% B to 15% B (15 min), 15% B to 100% B (10 min), 100% B (2.5 min), 100% B to 0% B (2.5 min). Monitoring was performed at 370 nm at a flow rate of 6 ml/min. The eluates were evaporated *in vacuo* to dryness, and the residue (16.1 mg) was stored at −80 °C in a nitrogen atmosphere.

Acid hydrolysis

The purified sample (1.6 mg) was hydrolyzed under reflux in a nitrogen atmosphere (100 °C, 120 min) using 10 ml of 2 N hydrochloric acid. After cooling, the solution was applied on an activated 1000 mg C_{18}-Sep-Pak cartridge and the latter was rinsed with 25 ml water. The carbohydrate containing effluent was collected and final purification was performed by HPLC (Phenomenex, Rezex RCM Monosaccharide, 300 × 7.8 mm; water as mobile phase, 0.55 ml/min, 80 °C). D-galactose (0.4 mg) was identified by ^{1}H NMR and optical rotation ($[\alpha]_D^{25} + 85$) in comparison with an authentic sample ($[\alpha]_D^{25} + 83$) obtained from Sigma. The anthocyanidin **2** was eluted from the C_{18}-Sep-Pak cartridge with acidified MeOH (0.01% hydrochloric acid) and the eluate was evaporated to dryness *in vacuo*.

*7-O-Methylcyanidin 3-O-β-D-galactopyranoside (**1**)*

Bluish-red amorphous powder. – $[\alpha]_D^{25} - 237$ ($c = 0.11$, 0.1% TFA). – UV/vis (MeOH, 0.1% HCl): $\lambda_{max}(\lg\varepsilon) =$ 282 nm (3.98), 523 nm (4.13). – IR (ATR): $\tilde{\nu} = 3109$, 1673, 1327, 1280, 1181, 1129 cm^{-1}. – ^{1}H NMR (500.14 MHz, D_2O, 1% TFA): $\delta = 3.63$ (s, 3 H, OMe), 3.70 (dd, $J = 9.6$, 4.7 Hz, 1 H, 3"-H), 3.70 (m, 2 H, 6"-H), 3.77 (dd, $J = 9.6$, 7.9 Hz, 1 H, 2"-H), 3.77 (bt, $J = 8.5$ Hz, 1 H, 5"-H), 3.91 (bd, $J = 3.9$ Hz, 1 H, 4"-H), 5.00 (d, $J = 7.6$ Hz, 1 H, 5-H), 6.13 (bs, 1 H, 6-H), 6.19 (bs, 1 H, 8-H), 6.20 (d, $J = 7.8$ Hz, 1 H, 5'-H), 6.91 (bs, 1 H, 2'-H), 7.22 (bd, $J = 7.9$ Hz, 1 H, 6'-H), 8.16 (bs, 1 H, 4-H). - ^{13}C {^{1}H} NMR (75.42 MHz, D_2O, 1% TFA): $\delta = 58.7$ (OMe), 62.7 (C-6"), 70.1 (C-4"), 72.0 (C-2"), 74.7 (C-3"), 77.9 (C-5"), 94.0 (C-8), 103.4 (C-1"), 103.4 (C-6), 113.1 (C-10), 118.1 (C-2'), 118.3 (C-5'), 120.1 (C-1'), 128.7 (C-6'), 134.5 (C-4), 145.5 (C-3), 146.3 (C-3'), 155.5 (C-4'), 155.8 (C-9), 157.5 (C-5), 161.2 (C-2), 170.6 (C-7). - ESI(+)-MS^2: *m/z* 463 [M$^+$], 301 [M$^+$-galactopyranosyl+H]. - HRFABMS: *m/z* 463.1203 (calcd. for $C_{22}H_{23}O_{11}$: 463.1239).

*7-O-Methylcyanidin (**2**)*

^{1}H NMR (500.14 MHz, CD_3OD, 5% TFA): $\delta = 4.03$ (s, 3 H, OMe), 6.68 (d, $J = 2.1$, 1 H, 6-H), 7.04 (d, $J = 8.7$ Hz, 1 H, 5'-H), 7.15 (dd, $J = 0.8$, 2.0 Hz, 1 H, 8-H), 8.19 (d, $J = 2.3$ Hz, 1 H, 2'-H), 8.31 (dd, $J = 2.2$, 8.7 Hz, 1 H, 6'-H), 8.61 (d, $J = 0.7$ Hz, 1 H, 4-H). – ^{13}C NMR (indirectly determined by GHSQC and GHMQC at 500.14 MHz): $\delta = 57.2$ (OMe), 92.3 (C-8), 102.9 (C-6), 114.0 (C-10), 117.3 (C-5'), 118.2 (C-2'), 121.8 (C-1'), 127.6 (C-6'), 133.3 (C-4), 147.4 (C-3'), 147.7 (C-3), 155.7 (C-4'), 156.6 (C-9), 157.2 (C-5), 163.1 (C-2), 169.2 (C-7). – ESI(+)-MS^2: *m/z* 301 [M$^+$].

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