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Research Article

Synthesis of [4-^{14}C]-pelargonidin chloride and [4-^{14}C]-delphinidin chloride

Michael Kraus, Ellen Biskup, Elke Richling and Peter Schreier*
Chair of Food Chemistry, University of Wuerzburg, Am Hubland, D-97074 Wuerzburg, Germany

Summary
The synthesis of [4-^{14}C]-pelargonidin chloride and [4-^{14}C]-delphinidin chloride via [formyl-^{14}C]-2-(benzoyloxy)-4,6-dihydroxybenzaldehyde, ω,4-diacetoxyacetophenone and ω,3,4,5-tetraacetoxyacetophenone is described. The first step comprised labelling of the carbonyl group of 2-(benzoyloxy)-4,6-dihydroxybenzaldehyde, verifying that the coupling with ω,4-diacetoxyacetophenone or ω,3,4,5-tetraacetoxyacetophenone under hydrogen chloride atmosphere resulted in the formation of [4-^{14}C] labelled anthocyanidins. Copyright © 2006 John Wiley & Sons, Ltd.

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Introduction
Anthocyanidins are polyphenolic substances belonging to the flavonoids, a large and widespread group of secondary metabolites of higher plants.[1] The six major aglycons occurring in nature are cyanidin, delphinidin, malvidin, pelargonidin, peonidin and petunidin. The structure of the aglycon is the so-called flavylium cation as shown in Figure 1.

Usually, anthocyanidins occur in nature in their glycosylated forms, i.e. the anthocyanins. These water soluble substances are responsible for the characteristic red, blue or purple pigments of many flowers, seeds, leaves, fruits and vegetables.[2] Anthocyanins are also widely distributed in the human

*Correspondence to: Peter Schreier, Chair of Food Chemistry, University of Wuerzburg, Am Hubland, D-97074 Wuerzburg, Germany. E-mail: schreier@pzlc.uni-wuerzburg.de

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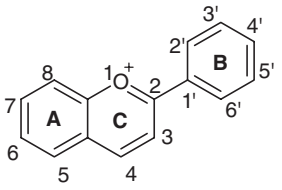

		position							
		3	5	6	7	3'	4'	5'	color at pH < 2
pelargonidin	**1**	OH	OH	H	OH	H	OH	H	orange
delphinidin	**2**	OH	OH	H	OH	OH	OH	OH	violet
cyanidin		OH	OH	H	OH	OH	OH	H	red
peonidin		OH	OH	H	OH	OMe	OH	H	red
malvidin		OH	OH	H	OH	OMe	OH	OMe	violet
petunidin		OH	OH	H	OH	OMe	OH	OH	violet

Figure 1. Structures and colours of major anthocyanin aglycons (anthocyanidins) investigated at pH < 2

diet; consumption has been estimated to range from 180 to 215 mg/day in the USA[3–6] and around 82 mg/day in Finland.[7]

Anthocyanins have been associated with potentially beneficial effects on various diseases. In several animal studies, chemopreventive properties of anthocyanins have been postulated,[8–10] raising the question towards the underlying mechanism of action. Recently, delphinidin and malvidin were found to be potent inhibitors of the epidermal growth factor receptor (EGFR)[11] and of 3',5'-cyclic adenosine monophosphate (cAMP) phosphodiesterases.[12]

Thus, as physiologically active compounds anthocyanins and their aglycons are attractive substances for the production of so-called functional foods.[13] In order to perform risk/benefit evaluations, further studies, in particular, comprehensive *in vivo* investigations about their metabolism, bioavailability, distribution and excretion are required. For such purposes sufficient amounts of anthocyanidins, preferably also in their ^{13}C and ^{14}C labelled form, are required. In this paper, we describe the synthesis of [4-^{14}C]-pelargonidin and [4-^{14}C]-delphinidin chloride based on the preparation of their unlabelled forms.

Results and discussion

The anthocyanidins, [4-^{14}C]-pelargonidin chloride (**1a**) and [4-^{14}C]-delphinidin chloride (**2a**) were synthesized using 2-(benzoyloxy)-4,6-dihydroxybenzaldehyde (**4, 4a**) (Scheme 1). In the anthocyanidins, the 2,4,6-trihydroxybenzaldehyde

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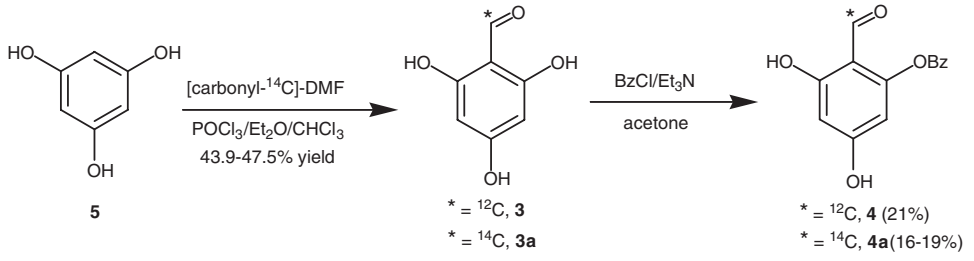

Scheme 1.

(**3**) moiety represents the A-ring; the carbonyl carbon atom becomes the 4-position in the molecule. The B-ring derives from ω,4-diacetoxyacetophenone (**6**) and ω,3,4,5-tetraacetoxyacetophenone (**7**) in **1** and **2**, respectively. In order to introduce the carbon-14 atom, a Vilsmeyer reaction was carried out between phloroglucinol (1,3,5-trihydroxybenzene, **5**) and [formyl-^{14}C]-dimethylformamide to yield **3a** in 43.9 and 47.5% yield (two separate runs) after purification by reverse phase high performance liquid chromatography (RP18-HPLC).[14] Acylation of **3** and **3a** with benzoyl chloride/EtN_3 followed by HPLC (on silica gel) yielded 2-(benzoyloxy)-4,6-dihydroxybenzaldehyde in 21% (**4**) and 16–19% (**4a**, two separate runs), respectively.[15] This reaction led to a mixture of mono- and di-benzoylated products. Because of the small scale, product purification was performed by RP18-HPLC.

The preparation of unlabelled and labelled pelargonidin chloride (**1**, **1a**) and delphinidin chloride (**2**, **2a**) were performed by condensation of **4**/**4a** and ω,4–diacetoxyacetophenone (**6**) or ω,3,4,5-tetraacetoxyacetophenone (**7**), respectively**.** Anisole was acylated by reaction with a mixture of chloroacetyl chloride/$AlCl_3$; additional $AlCl_3$ was given to effect conversion of the methyl ether **8** to the phenol **9** (yield 20%).[16] Reaction of **9** with HOAc/Et_3N, followed by acylation with acetic anhydride yielded **6** via **10** in 30% yield (two steps) (Scheme 2).[17] Condensation of **6** with **4** in the presence of anhydrous HCl/CH_3OH/EtOAc, followed by deprotection with KOH/aqueous methanol yielded pelargonidin chloride (**1**). The anthocyanidin **1** was crystallized as its HCl salt, converted to the corresponding picrate and finally recrystallized as its HCl salt (yield 40%).[18] Reaction of **6** with **4a** in the same manner, followed by purification using RP18-HPLC yielded [4-^{14}C]-pelargonidin chloride (**1a)** with a specific activity of 33.6 μCi/mg; 0.2 mg was obtained (3.8%).

For the syntheses of **2** and **2a**, first of all**,** acetylation of gallic acid (3,4,5-trihydroxybenzoic acid) (**11)** provided 3,4,5-triacetoxybenzoic acid (**12**) in 79% yield.[19] Conversion of **12** to its acyl chloride **13** (yield, 91%) and subsequent reaction of **13** with diazomethane yielded the diazoketone **14** (yield, 81%).[18,20] Direct conversion of **14** to **7** by reaction with acetic acid was unsuccessful;

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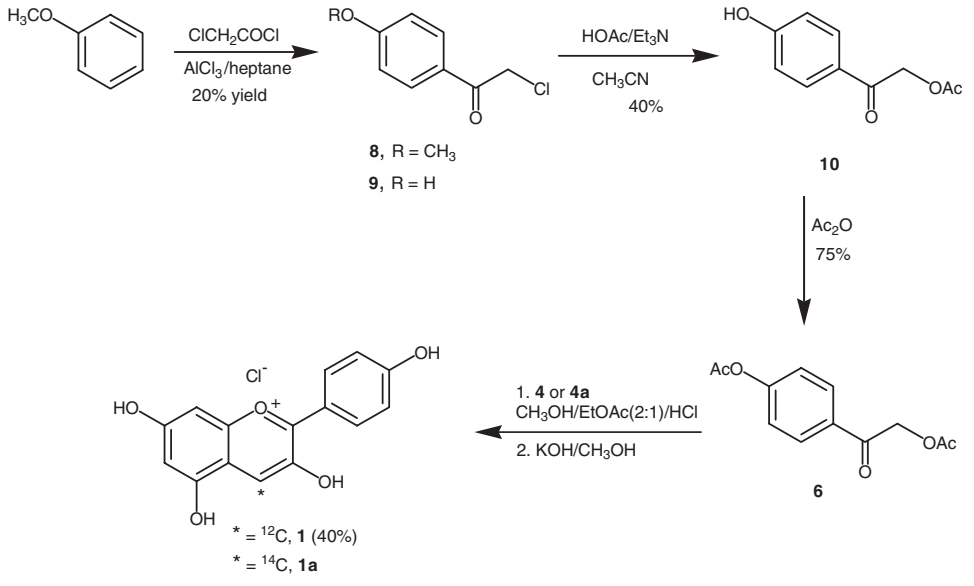

Scheme 2.

reaction of **14** with HCl/Et_2O[21] yielded ω-chloro-3,4,5-triacetoxyacetophenone (**15)** in 87% yield. Subsequent reaction of **15** with $KOAc/Ac_2O/HOAc$ yielded **7** in 75% yield.[22] Delphinidin chloride (**2**) and [4-^{14}C]-delphinidin chloride (**2a**) were prepared by reaction of **7** with **4** (or **4a**) using anhydrous HCl in EtOH/EtOAc followed by reaction with KOH. Delphinidin chloride (**2)** was isolated in 42% yield by crystallization from EtOH/HCl;[20] **2a** was isolated directly from the reaction mixture (along with its benzoate ester **16).** Both were purified by RP-18 HPLC; treatment of **16** with KOH yielded additional **2a** which was combined with the original **2a** isolated directly. The combined yield was 5.5%; the specific activity was 30.4 Ci/mg (Scheme 3).

Experimental

Reagents

[Formyl-^{14}C]-dimethylformamide with a specific activity of 53 mCi/mmol was purchased from American Radiolabelled Chemicals ARC, Inc. (St. Louis, MO, USA) and was used without further purification. 1,3,5-Trihydroxybenzene (phloroglucinol), N,N-dimethylformamide (N,N-DMF), benzoyl chloride, 3,4,5-trihydroxybenzoic acid (gallic acid), phosphorus oxychloride ($POCl_3$) and 1 N hydrogen chloride (in diethyl ether) were purchased from Fluka (Deisenhofen, Germany). Triethylamine, potassium acetate, trifluoroacetic acid and Diazald^{TM} were from Sigma Aldrich (Steinheim, Germany). All chemicals were of analytical grade (purity > 98%). The solvents for

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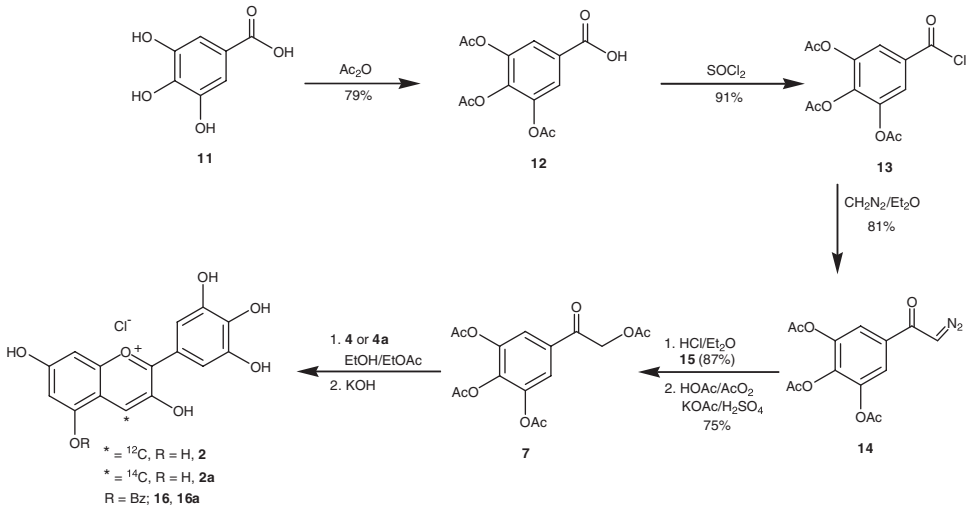

Scheme 3.

synthesis were redistilled and stored on molecular sieve (5 Å) before use. Acetonitrile (HPLC grade) was purchased from Fisher Scientific (Loughborough, UK).

Analyses

The HPLC instrumentation comprised two Knauer 64 pumps, a Knauer mixing chamber and a Knauer UV/VIS-Detector (Knauer, Berlin, Germany). A Knauer Eurospher 100 C18 column (250 × 4.6 mm, 5 µm) was used. The mobile phase consisted of aqueous 0.1% trifluoroacetic acid (A) and acetonitrile (B) (v/v). The gradient applied was 1–99% B in 45 min at a flow rate of 1 ml/min and 25 µl- injection volumes were employed.

The purity of the unlabelled substances was controlled by HPLC diode array detector (DAD). The HPLC-DAD system was a Hewlett-Packard 1100 HPLC gradient pump and a Hewlett-Packard 1100 photodiode array detector (Waldbronn, Germany), equipped with a Wisp 710b autosampler (Waters, Eschborn, Germany). Data acquisition and evaluation were performed with Chemstation^{TM} software (Hewlett-Packard, Waldbronn, Germany). A Knauer Europher 100 C18 column (250 × 4.0 mm, 5 µm) was used. The mobile phase consisted of aqueous 0.1% formic acid (A) and acetonitrile (B) (v/v). The gradient applied was 1–99% B in 40 min at a flow rate of 1 ml/min and 20 µl-injection volumes were employed.

The specific activity of **1a** and **2a** was determined by liquid scintillation counting (LSC) using a Rackbeta 1214 LKB Pharmacia scintillation counter (Wallac, Freiburg, Germany) with 15 ml vessels of polyethylene (Packard

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Bioscience, Billerica, MA, USA) and Rotiszint Ecoplus® (Roth, Karlsruhe, Germany) as scintillation cocktail. Data evaluation was performed by Ultro-Term® Software (Wallac, Freiburg, Germany).

^{1}H-NMR spectra were recorded on a Bruker AVANCE 400 NMR spectrometer (Rheinstetten, Germany). As solvents DMSO-d_6, methanol-d_4 and $CDCl_3$ (stored on mol sieve, 5 Å) were used. Data evaluation was performed by Mestre-C 4.4.1.0 software.

Mass spectrometrical data were obtained by LC-ESI-MS/MS and GC-MS analyses. LC-ESI-MS/MS data were recorded with a Finnigan TSQ 7000 apparatus (Finnigan MAT, Bremen, Germany) with an electrospray ionisation interface (ESI). Aqueous 0.1% acetic acid (A) and acetonitrile (B) (v/v) was used as mobile phase in an isocratic gradient (50:50). An Applied Biosystems 140B pump was used. The capillary temperature was 200°C and the spray capillary voltage was set to 3.2 kV. Nitrogen served as both sheath (70 psi) and auxiliary gas (10 units). The mass spectrometer was operated in the full scan mode, m/z 20–400, with a total scan duration of 1.0 s. MS/MS experiments were performed at a collision energy of 20–35 eV, with argon (2.0 mTorr) serving as collision gas. The multiplier voltage was set to 1300 V.

GC-MS was carried out using a Fisons Instrument GC 8060 (Thermo Electron, Dreieich, Germany) gas chromatograph with split injection (220°C; 1:20) directly coupled to a MD 800 mass spectrometer (Thermo Electron, Dreieich, Germany). The GC was equipped with a HP 5 capillary column (30 m × 0.25 mm, d_f = 0.25 µm) and a temperature program running from 60°C to 300°C at 5°C/min with a gas flow of 2 ml/min helium under constant pressure was used. Temperature of connecting parts was 220°C, electron energy for the EI mass spectra was 70 eV. Data evaluation was performed with Xcalibur™ software (Thermo Electron, Dreieich, Germany).

*2-(Benzoyloxy)-4,6-dihydroxybenzaldehyde (**4**)*

To a solution of 10 g (0.065 mol) 2,4,6-trihydroxybenzaldehyde **3** in 150 ml dry acetone kept under nitrogen atmosphere and cooled at 0°C, each a solution of 5 g (0.049 mol) triethylamine and 6.8 g (0.048 mol) benzoyl chloride in 30 ml dry acetone was added dropwise within 30 min. After subsequent stirring for half an hour at room temperature 50 g of silica gel (0.063–0.2 mm, Merck, Darmstadt, Germany) was added and the mixture was evaporated to dryness under reduced pressure. The dry yellow powder was applied to a 5.5 × 35 cm glass column filled with silica gel (0.063–0.2 mm, Merck, Darmstadt, Germany) and preconditioned with benzene. Elution with 1 l benzene gave fraction 1. The second fraction was eluted using 1 l of a chloroform/benzene (1 + 1) mixture; in this fraction the dibenzoylated product was

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achieved. In fraction 3, eluted with 2 l of a chloroform/ethyl acetate (1 + 1) mixture, 3.4 g of 2-(benzoyloxy)-4,6-dihydroxybenzaldehyde (**4**) was isolated (3.6 g, 21% yield). Owing to the high purity of the product (HPLC-DAD control, 99%), no recrystallization process was necessary HY.[15] ^{1}H-NMR: DMSO-d_6, 400 MHz: δ 6.27 (Ar, *d*, 2.1 Hz , 2H), 6.32 (Ar, *d*, 2.1 Hz , 2H), 7.59 (Ar, *t*, 7.6 Hz, 1H), 7.74 (Ar, *t*, 7.4 Hz, 1H), 8.11 (Ar, *d*, 7.2 Hz, 1H), 10.00 (CHO, *s*, 1H), 11.01 (OH, *s*, 1H), 11.38 (OH, *s*, 1H). LC-ESI$_{neg}$-MS/MS (%) [20 eV]: 257.0 (100), 213.0 (20), 187.0 (58), 169.0 (16), 145.0 (13), 143.0 (24), 119.0 (19), 111.1 (24).

*Synthesis of pelargonidin chloride (***1***)*

*ω-Chloro-4-hydroxyacetophenone (***9***).* To a solution of 30 g (0.28 mol) anisole and 36 g (0.32 mol) chloroacetyl chloride in 500 ml *n*-heptane was added stepwise 45 g (0.34 mol) $AlCl_3$ and the mixture was subsequently heated to 35°C for 90 min. During the following 30 min, again 45 g $AlCl_3$ (0.34 mol) was added and the solution heated for 2.5 h. The solvent was removed under reduced pressure and ice was put to the residue. The solution was then acidified with 200 ml of concentrated hydrochloric acid. After extraction with 250 ml of diethyl ether for three times, the ether fraction was neutralized using 200 ml of a 15% ammonium carbonate solution. After this, the organic phase was extracted with 250 ml of a 10% sodium carbonate solution. The water phase was acidified and extracted with diethyl ether for several times. The ether phase was evaporated and a yellow oil was obtained. The yellow ω-chloro-4-hydroxyacetophenone (**9**) crystallized from 25% aqueous ethanol solution (9.5 g, 20% yield).[17] ^{1}H-NMR: DMSO-d_6, 400 MHz: δ 5.02 (CH_2, *s*, 2H), 6.84 (Ar, *d*, 8.8 Hz, 2H), 7.84 (Ar, *d*, 8.7 Hz, 2H), 10.48 (OH, *s*, 1H). LC-ESI$_{neg}$-MS/MS (%) [20 eV]: 168.7 (100), 132.7 (52), 126.8 (7), 122.9 (27), 104.8 (32), 77.0 (15), 58.6 (4), 34.9 (93).

*ω-Acetoxy-4-hydroxyacetophenone (***10***)*

A solution of 10 g (0.06 mol) ω-chloro-4-hydroxyacetophenone (**9**) and 7.7 ml acetic acid in 100 ml anhydrous acetonitrile was cooled on ice. Step by step, 11.2 g (0.11 mol) of triethylamine was added and the mixture was heated under reflux for 3 h. The solution was diluted with diethyl ether and washed with hydrochloric acid, water and sodium chloride solution. The organic phase was dried over sodium sulphate. After evaporating the solvent under reduced pressure, the solid of **10** was crystallized in water (4.5 g, 40% yield).[21] ^{1}H-NMR: DMSO-d_6, 400 MHz: δ 2.15 (CH_3, *s*, 3H), 5.40 (CH_2, *s*, 2H), 7.13 (Ar, *d*, 8.8 Hz, 2H), 7.91 (Ar, *d*, 8.8 Hz, 2H), 10.31 (OH, *s*, 1H). LC-ESI$_{neg}$-MS/MS (%) [20 eV]: 192.9 (12), 150.9 (100), 132.8 (9), 122.7 (14), 106.8 (12), 104.8 (21), 58.8 (35).

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*ω,4-Diacetoxyacetophenone (**6**)*

ω-Acetoxy-4-hydroxyacetophenone (**10**) (4.9 g, 0.025 mol) solved in 20 ml acetic anhydride was heated for 2 h under reflux at 100°C and then poured in 100 ml of boiling water. After boiling for a short time, subsequent cooling down led to crystallization of ω,4-diacetoxyacetophenone (**6**) (4.0 g, 75% yield).[22] ^{1}H-NMR: DMSO-d_6, 400 MHz: δ 2.15 (CH_3, *s*, 3H), 2.31 (CH_3, *s*, 3H), 5.46 (CH_2, *s*, 2H), 7.33 (Ar, *d*, 8.8 Hz, 2H), 8.03 (Ar, *d*, 8.8 Hz, 2H). LC-ESI_{neg}-MS/MS (%) [20 eV]: 237 (7), 194.9 (60), 152.8 (100), 134.7 (13), 106.8 (8).

*Pelargonidin chloride (**1**)*

2-(Benzoyloxy)-4,6-dihydroxybenzaldehyde (**4**) (2.03 g, 7.86 mmol) and ω,4-diacetoxyacetophenone (**6**) (2.03 g, 8.6 mmol) were dissolved in 30 ml methanol/ethyl acetate (2 + 1) mixture. For 1 h, dry hydrogen chloride gas was bubbled into the solution and the mixture was stirred at room temperature for 24 h. After removing of the solvent by centrifugation, the purple residue of 5-(benzoyloxy)pelargonidin chloride was suspended in 30 ml of a basic methanol/water solution (0.25 g potassium hydroxide in 5 ml methanol/water 1:1) under ice cooling and stirred for 1 h at 0–4 °C. The blue solution was acidified with 16.5 ml of concentrated hydrochloric acid. The colour changed to purple again and **1** crystallized in brownish crystals that were recovered after centrifugation. For recrystallization via the picrate it was solved in 1% HCI solution containing 50% ethanol, added to a solution of picric acid in 50% aqueous ethanol and heated to 60°C for 2 min. The picrate crystallized over night in a freezer. The crystals were dried and washed with dry diethyl ether. To regenerate **1,** methanol, saturated with hydrogen chloride gas, was added to the picrate. Dry diethyl ether was added in excess (~6 fold of methanol) and **1** crystallized in crude crystals. The crystallization process was repeated leading to 967 mg (40% yield) pelargonidin chloride **1** with 95% purity (HPLC-DAD control). ^{1}H-NMR: DMSO-d_6,400 MHz: δ 6.86 (Ar, *d*, 1.8 Hz, 1H), 6.97 (Ar, *d*, 1.4 Hz, 1H), 7.11 (Ar, *d*, 9.1 Hz, 2H), 8.54 (Ar, *d*, 9.1 Hz, 2H), 8.84 (CH, *s*, 1H), 11.31 (OH, *s*, 1H), 12.14 (OH, *s*, 1H), 12.20 (OH, *s*, 1H), 12.35 (OH, *s*, 1H). LC-ESI_{neg}-MS/MS (%) [20 eV]: 270.9 (93), 196.8 (19), 168.9 (17), 158.9 (8), 152.9 (8), 144.8 (16), 140.7 (19), 120.8 (100), 92.9 (10).

*Synthesis of delphinidin chloride (**2**)*

*3,4,5-Triacetylbenzoic acid (**12**).* A solution of 50 g (0.3 mol) 3,4,5-trihydroxybenzoic acid (gallic acid) (**11**) in 200 ml acetic anhydride was heated under reflux at 110°C for 3 h. The solution was then poured into 1 l hot water. During cooling 3,4,5-triacetylbenzoic acid (**12**) crystallized. After filtering and washing with water, the white crystals were dried over silica gel. The yield was

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62 g (79%).[18] ^{1}H-NMR: DMSO-d_6, 400 MHz: δ 2.30 (CH_3, *s,* 6H), 2.33 (CH_3, *s*, 3H), 7.75 (Ar, *s*, 2H), 13.38 (COOH, *s*, 1H). LC-ESI_{neg}-MS/MS (%) [20 eV]: 296.1 (3), 251.8 (50), 209.7 (100), 168.0 (36), 166.7 (79), 149.4 (5), 125.0 (5), 59.0 (8).

*3,4,5-Triacetylbenzoic acid chloride (***13***).* 3,4,5-Triacetylbenzoic acid (**12**) (25 g, 0.084 mol) was refluxed with 30 ml of thionyl chloride at 85°C for 1 h. The excess of thionyl chloride was removed by evaporating under reduced pressure. After drying, the pale yellow residue was washed with diethyl ether. The raw product **12** was recrystallized from xylene. The yield was 23.8 g (91%).[18] ^{1}H-NMR: DMSO-d_6, 400 MHz: δ 2.31 (CH_3, *s*, 6H), 2.32 (CH_3, *s*, 3H), 7.89 (Ar, *s*, 2H). GC-MS (70 eV): 268 (34), 237 (18), 227 (19), 226 (71), 209 (22), 185 (28), 184 (99), 153 (51), 152 (19), 43 (100).

*ω-Diazo-3,4,5-triacetoxyacetophenone (***14***).* Diazomethane was generated from an etheric solution of Diazald™ (13.6 g) in the presence of 13 g potassium hydroxide dissolved in 13 ml water and 45 ml ethanol. 3,4,5-Triacetylbenzoic acid chloride (**13**) (5 g, 0.016 mol), dissolved in 12 ml chloroform and cooled to −10°C, was poured step by step within 30 min into the diazomethane solution (−10°C), yielding a yellow powder of ω-diazo-3,4,5-Triacetoxyacetophenone (**14**) which was collected and washed with diethyl ether. The product (4.2 g, 81% yield) was stored in the freezer at −24°C.[20] ^{1}H-NMR: $CDCl_3$, 400 MHz: δ 2.30 (CH_3, *s*, 6H), 2.31 (CH_3, *s*, 3H), 5.83 (CH, *s*, 1 H), 7.52 (ar, *s*, 2H). Owing to instability no mass data are available.

*ω-Chloro-3,4,5-triacetoxyacetophenone (***15***).* ω-Diazo-3,4,5-triacetoxyacetophenone (**14**) (3.0 g, 9.77 mmol), dissolved in 16 ml dioxane was cooled to 10°C. Then 12 ml of 1 N hydrogen chloride solved in diethyl ether was added to the solution and the mixture was stirred for 30 min at 10°C. After a strong development of nitrogen, the diethyl ether was removed in a stream of nitrogen; dioxane was evaporated under reduced pressure. The pale yellow residue was solved in dichloromethane and washed with water. The organic phase was dried with magnesium sulphate and evaporated under vacuum. The residue was dissolved in ethyl acetate/petrol ether (1/1, v/v). The solution was diluted with petrol ether and **15** was obtained as a fine crystalline powder (2.6 g, 87% yield).[21] ^{1}H-NMR: DMSO-d_6, 400 MHz: δ 2.32 (CH3, *s*, 6H), 2.34 (CH3, *s*, 3H), 5.17 (CH2, *s*, 2H), 7.87 (Ar, *s*, 2H). LC-ESI_{pos}-MS/MS (%) [20 eV]: 329.9 (7), 287.7 (12), 245.8 (100), 203.8 (30).

*ω,3,4,5-Tetracetoxyacetophenone (***7***).* A mixture of 1 g (5.4 mmol) ω-chloro-3,4,5-triacetoxyacetophenone (**15**), 3.9 ml (42 mmol) acetic anhydride, 2 µl concentrated sulphuric acid, 3.9 ml glacial acetic acid and 1.53 g potassium acetate was kept at 100°C under reflux for 3 h and then poured into a mixture

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of water/ice. The precipitate was filtered and crystallized from ethyl acetate/cyclohexane (1:1). The yield of **7** was 0.8 g (75% yield).[22] ^{1}H-NMR: DMSO-d_6, 400 MHz: δ 2.15 (CH_3, *s*, 3H), 2.32 (CH_3, *s*, 6H), 2.35 (CH_3, *s*, 3H), 5.43 (CH_2, *s*, 2H), 7.85 (Ar, *s*, 2H). LC-ESI_{pos}-MS/MS (%) [20 eV]: 353.1 (4), 310.8 (7), 268.9 (100), 226.9 (27).

Delphinidin chloride (**2**). In a solution of 0.25 g (0.71 mmol) ω-3,4,5-tetraacetoxyacetophenone (**7**) and 0.2 g (0.78 mmol) 2-(benzoyloxy)-4,6-dihydroxybenzaldehyde (**4**) in 5 ml absolute ethanol and 5 ml ethyl acetate, kept under argon, dry hydrogen chloride gas was applied for 1 h. After 24 h brownish crystals of 5-(benzoyloxy)-delphinidin chloride were obtained by centrifugation (5.000 *g*). The cleavage of the benzoyl group took place by adding 2.5 ml of an oxygen-free aqueous solution of 10% sodium hydroxide at 0°C under argon atmosphere. After keeping at 0–4°C for 15 min the blue solution was stored for 4 h at room temperature. After adding 5 ml of ethanol and 5 ml of concentrated hydrochloric acid, the mixture was kept for 4 days at 4°C. Then, 10 ml of concentrated hydrochloric acid was added and the mixture was stored for two further days at 4°C leading to the crystallization of **2**.[20] After centrifugation, the residue was solved in a small amount of methanol, saturated with hydrogen chloride gas. By addition of absolute diethyl ether, in total, 220 mg (42% yield) of **2** was obtained. ^{1}H-NMR: methanol-d_6, 400 MHz: δ 5.90 (Ar, δ, 2.2Hz, 1H), 5.94 (Ar, *d*, 2.2 Hz, 1H), 6.14 (CH, *s*, 1H), 6.62 (Ar, *s*, 2H). LC-ESI_{pos}-MS/MS (%) [20 eV]: 303.0 (66), 257.0 (31), 229.0 (100), 201.0 (24), 173.0 (36), 163.2 (12), 153.0 (42), 149.8 (15), 148.9 (11), 145.0 (22), 127.1 (17), 125.0 (20), 121.0 (22), 107.1 (10), 79.1 (11), 69.1 (10).

[Formyl-^{14}C]-2,4,6-trihydroxybenzaldehyde (**3a**). 2,4,6-Trihydroxybenzene (**5**) (30 mg, 0.24 mmol) was dissolved in an 1.5 ml diethyl ether/chloroform (2:1) mixture, dimethylformamide (18.1 µl; 0.21 mmol) unlabelled and [formyl-^{14}C]-dimethylformamide, 2500 µCi) were added and mixed under ice cooling. After solvation, 22 µl phosphoroxychloride ($POCl_3$) was added. Then a nitrogen atmosphere was created and the mixture was stirred on ice for 1 h. After stirring for half an hour at room temperature, 11 µl of $POCl_3$ was added. Under nitrogen atmosphere and light protection, the mixture was heated at 60°C for 4 h. After removal of the solvent, ice was added under sonification. The solution was adjusted to pH 6 using sodium hydroxide and then lyophilized. To the residue 4 ml methanol was added and the mixture, after 2 min of sonification, centrifuged (5000 g, 10 min). The supernatant was purified by RP18-HPLC yielding 17.4 mg (43.9%) and 15.9 mg (47.5%) (two separate runs) of the ^{14}C-labelled 2,4,6-trihydroxybenzaldehyde **3a**.

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*[Formyl-^{14}C]-2-(benzoyloxy)-4,6-dihydroxybenzaldehyde (***4a***)*. The obtained **3a** was dissolved in 700 µl anhydrous acetone and 9 µl anhydrous triethylamine and 7.5 µl anhydrous benzoyl chloride were added to the solution which was stored on ice. After stirring under nitrogen atmosphere for 1 h at 0–4°C and removing of the solvent, the residue was solved in 2 ml methanol. Purification by RP18-HPLC led to 4.2 mg/5 mg (yields 16%/18.9%, two separate runs) of [formyl-^{14}C]-2-(benzoyloxy)-4,6-dihydroxybenzaldehyde **4a**.

*[4-^{14}C]-Pelargonidin chloride (***1a***)*. Five mg of [formyl-^{14}C]-2-(benzoyloxy)-4,6-dihydroxybenzaldehyde (**4a**) (0.018 mmol, first preparation) and 5 mg (0.020 mmol) ω,4-diacetoxyacetophenone (**6**) (molar ratio of 1:1.1) were solved in 500 µl methanol/ethyl acetate. For 1 h, dry HCI gas was transfered into the solution with a fused silica capillary. Afterwards, the mixture was stirred for 24 h at room temperature. After removing the solvent, the purple residue was suspended in 80 µl of a basic methanol/water solution (0.25 g potassium hydroxide in 5 ml methanol/water 1:1) under ice cooling and stirred for 1 h at 0–4°C. The blue solution was acidified with 51 µl concentrated hydrochloric acid. The colour changed to purple again. After freezing and lyophilizing, the residue was dissolved in methanol and injected for purification to RP18-HPLC. The pelargonidin chloride **1a** was detected at 520 nm. The yield of the ^{14}C-radiolabelled pelargonidin chloride was 0.2 mg (3.8%). The specific activity was 33.6 µCi/mg.

*[4-^{14}C]-Delphinidin chloride (***2a***)*. [Formyl-^{14}C]-2-(benzoyloxy)-4,6-dihydroxybenzaldehyde (**4a**) (4.7 mg, 0.018 mmol) and 7.1 mg (0.020 mmol) ω,3,4,5-tetracetoxyacetophenone (**7**) were dissolved in each of 100 µl ethanol and ethyl acetate. Under ice cooling the mixture was saturated with hydrogen chloride gas. The mixture was kept at room temperature for 24 h yielding [4-^{14}C]-delphinidin chloride (**2a**) and [4-^{14}C]-5-benzoyldelphinidin chloride (HPLC-DAD control). After removing of the solvent, the residue was dissolved in methanol, saturated with hydrogen chloride. The solution was subjected to preparative HPLC to yield **2a**. The remaining [4-^{14}C]-5-benzoyldelphinidin chloride was deprotected by resolving in 14 µl oxygen-free 10% sodium hydroxide solution and 100 µl ethanol at 0°C. The solution was stored under argon atmosphere for half an hour at room temperature. Then 20 µl concentrated hydrochloric acid was added. Under inert atmosphere the solution was stored for 4 days at 4°C. Purification was performed by HPLC to achieve the [4-^{14}C]-delphinidin chloride **2a**. At the end, 0.33 mg (5.5%) with a specific activity of 30.4 µCi/mg was obtained. ^{1}H-NMR: methanol-d_4, 400 MHz: δ 5.90 (Ar, *d*, 2.2 Hz, 1H), 5.94 (Ar, *d*, 2.2 Hz, 1H), 6.14 (CH, *s*, 1H), 6.62 (Ar, *s*, 2H).

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