Composition of an Anthocyan Concentrate from *Aronia melanocarpa* Elliot – X-ray Analysis of Tetraacetyl Parasorboside

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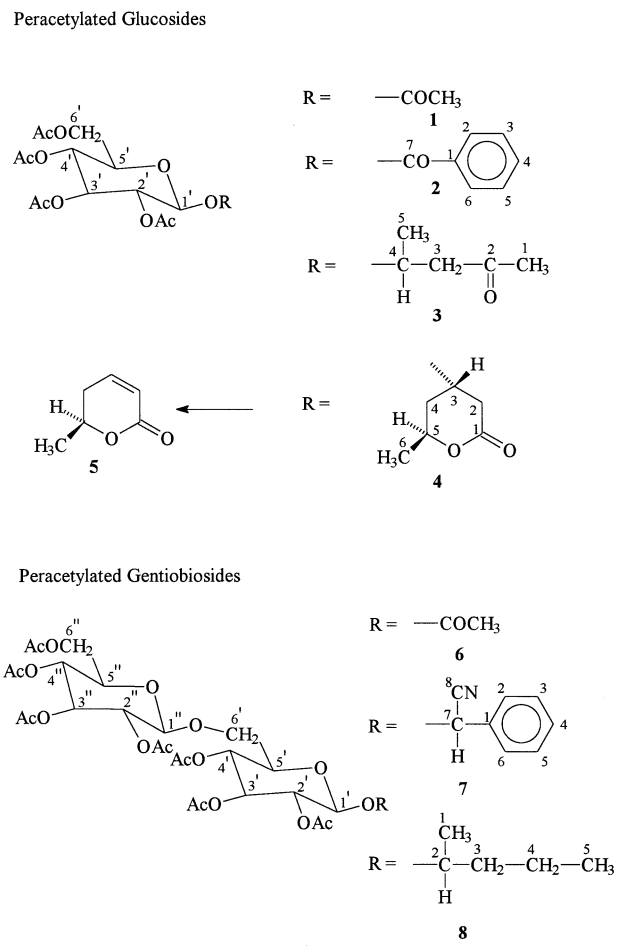
Following acetylation of an anthocyan concentrate from black chokeberries (fruits of *Aronia melanocarpa* Elliot = *Sorbus* melanocarpa Heynh., Rosaceae), which is used as a natural food colouring agent, hexa-*O*-acetyl-sorbitol, penta-*O*-acetyl-glucopyranose (**1**), tetra-*O*-acetyl-β-D-glucopyranosyl benzoate (**2**), and hepta-*O*-acetyl-amygdalin (**7**) were detected by comparison with authentic samples. The structures of the hitherto unknown compounds 4-*O*-(tetra-*O*-acetyl-β-D-glucopyranosyl)pentan-2-one (**3**) and hepta-*O*-acetyl (2-pentanyl)-β-D-gentiobioside (**8**) were elucidated from their ^{1}H,^{1}H- and ^{13}C,^{1}H-COSY spectra. The structure of tetra-*O*-acetyl parasorboside was confirmed by crystal structure analysis.

In many countries, the use of synthetic colouring agents in foodstuffs is prohibited and hence the use of natural colourings is of special interest. As natural red colourings, the anthocyans are undoubtedly the most important, since there is an abundance of fruits, such as blackcurrants, elderberries, some varieties of red grapes etc., from which they can be derived. Since the isolation of the anthocyans is very laborious, the industrial preparation of anthocyan concentrates is carried out by a simple method of adsorption onto Amberlite XAD. Consequently, these concentrates also contain other natural substances. In this paper, we report on the composition of an anthocyan concentrate[1] derived from black chokeberries (*Aronia melanocarpa* Elliot = *Sorbus melanocarpa* Heynh., Rosaceae), a largely unfamiliar fruit found in our region.

From 1000 ml of anthocyan concentrate from black chokeberries, 150 g of a dry residue was obtained after freeze-drying. Besides condensed tannins, the mixture contained the anthocyans cyanidin-3-galactoside and cyanidin-arabinoside as the principal constituents. Because these have been described previously in the literature[2], we did not investigate them any further. Since it is known that peracetates are readily separable by column chromatography with toluene/acetone as eluent and are more easily crystallized than the free compounds, 15 g of the anthocyan concentrate was acetylated with acetic anhydride/pyridine at room temperature. By means of ^{1}H- and ^{13}C-NMR spectroscopy and by comparison with authentic samples, hexa-*O*-acetylsorbitol, penta-*O*-acetyl-glucopyranose (**1**) and hepta-*O*-acetyl-amygdalin (**7**) could be directly identified.

Another crystalline peracetate was shown to be tetra-*O*-acetyl parasorboside (**4**). To date, **4** has only been isolated from the rowanberries of the mountain ash (*Sorbus aucuparia*, Rosaceae), as reported by Tschesche et al.[3] Compound **4** is the glucosidic preliminary stage of parasorbic acid (**5**). Since **5** is very volatile in steam[4], it could not have been



  1434–193X/98/0101–0189 $ 17.50+.50/0

present in the concentrate that we investigated. However, it can be supposed that parasorbic acid (**5**) occurs in the juice of black chokeberries, although it could not be detected by Handschack[5]. Whereas **5** shows an antibiotic effect at a concentration of >950 μg/ml, such an effect could not be confirmed for **4**[3]. Due to its side-effects, **5** is of no importance in medicine[5].

Three other peracetates could be identified by means of ^{1}H,^{1}H- and ^{1}H,^{13}C-COSY experiments as tetra-*O*-acetyl-β-D-glucopyranosyl benzoate (**2**), 4-*O*-(tetra-*O*-acetyl-β-D-glucopyranosyl)pentan-2-one (**3**), and hepta-*O*-acetyl 1-(2-pentanyl)-β-D-gentiobioside (**8**). The structure of **2** was further confirmed by comparison with the synthetic compound. Comparison of the NMR spectra with those of the peracetates **1**, **4**, **6**, and **7** was very informative. The ^{13}C-chemical shifts (Tables 1 and 2), which could be unambiguously assigned, are characteristic. Moreover, the MS determinations of the molar masses and the CH analyses correspond with the calculated values.

Table 1. ^{13}C-chemical shifts of the peracetylated glucosides (75.47 MHz, $CDCl_3$) (the chemical shifts of the CH_3CO groups are not given and are found at δ = 168−170 for C=O and at δ = 20 for CH_3)

C atom	**1**	**2**	**3**	**4**
1	–	128.45	22.09	ca. 170[a]
2	–	130.10	50.71	70.83
3	–	133.97	75.05	35.12
4	–	128.56	31.65	70.88
5	–	130.10	–	21.28
6	–	164.52	–	–
1′	91.62	92.34	101.98	99.12
2′	70.16	70.18	71.73	70.22
3′	72.70 [b]	72.81	73.19	72.66
4′	67.68	67.95	68.97	68.19
5′	72.64 [b]	72.69	72.01	72.37
6′	61.37	61.50	62.56	61.74

[a] The signal for C-1 is in the same region as the signals for $COCH_3$. – [b] The assignments may be interchanged.

Discussion

The anthocyan concentrate of black chokeberries, which is obtained by column chromatography on Amberlite XAD, consists mainly of condensed tannins and glycosides, whereas glucose and sorbitol are present in small quantities only. Non-glycosidic phenolic natural products could not be found. The occurrence of sorbitol is characteristic of pomaceous- and stone-fruit (Maloideae, Prunoideae), whereas soft fruit (Rosaoideae and berries of other plant families) does not contain sorbitol. Therefore, the identification of sorbitol[7] in a foodstuff can be taken as evidence of colouring with an anthocyan concentrate from pomaceous- or stone-fruit. Amygdalin (**7**) occurs in the seeds of many members of the Maloideae (Rosaceae)[8], to which the Sorbus species are classified. The storing of amygdalin in the seeds is accompanied by transfer to the fleshy fruits[9] so that the occurrence of **7** is not a special feature. However, since **7** is toxic[10], it would seem important that accurate physiological investigations are carried out. This is of special interest, since the most effective solitaire carcinogens (aflatoxins) and cocarcinogens (phorbolesters) are of plant origin. Characteristic of the genus of Sorbus is the occurrence of parasorboside (**4**), as a result of which the Aronia plant (*Aronia melanocarpa* Elliot) should more correctly, and chemotaxonomically, be named *Sorbus melanocarpa* Heynh.

Table 2. ^{13}C-chemical shifts of the peracetylated gentiobiosides (50.32 MHz, $CDCl_3$) (the chemical shifts of the CH_3CO groups are not given and are found at δ = 168−170 for C=O and at δ = 20 for CH_3)

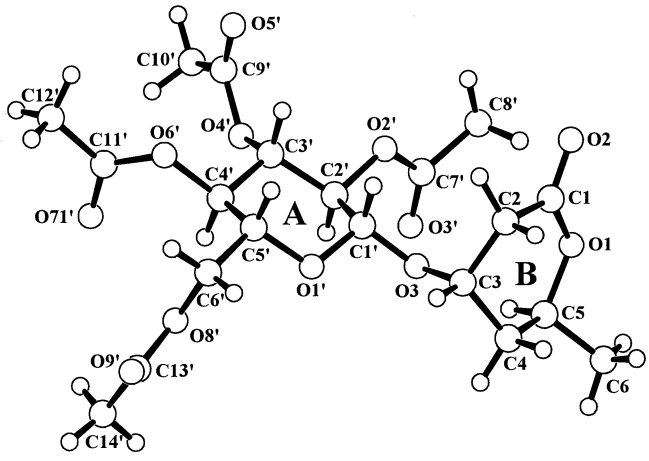
C atom	**6**[6]	**7**	**8**
1	–	132.16	21.80
2	–	129.28[a]	77.97
3	–	127.67[a]	38.85
4	–	130.41	18.29
5	–	127.67[a]	14.01
6	–	129.28[a]	–
7	–	67.89	–
8	–	117.15	–
1′	91.7	97.84	100.47
2′	70.4	70.88	69.19
3′	73.0	72.54	72.87
4′	68.6	68.69[b]	68.32[b]
5′	74.0	73.70	73.43
6′	67.6	67.73	68.13
1′′	100.7	100.47	100.91
2′′	71.1	71.34	71.19
3′′	72.9	72.41	71.99
4′′	68.6	68.15[b]	68.13[b]
5′′	72.1	71.89	71.67
6′′	62.0	61.73	61.85

[a][b] The assignments may be interchanged.

Crystal Structure Analysis of 4

The two six-membered rings of compound **4** (Figure 1) adopt chair conformations with folding angles of 54.7(2)° and 51.1(2)° (ring **A**: O1′/C2′/C3′/C5′ to O1′/C1′/C2′ and to C3′/C4′/C5′) and only 18.3(4)° and 48.2(3)° (ring **B**: O1/C2/C3/C5 to O1/C1/C2 and to C3/C4/C5). The flattening of one angle in ring **B** is caused by mesomeric effects between C1, O1 and O2, with bond lengths of 1.201(4) Å (C1−O2) and 1.322(4) Å (C1−O1).

Figure 1. Molecular structure of **4**[15]



With reference to the known configuration in ring **A**, we can assign (*S*) configuration at C5 bearing a methyl substituent (Figure 1) and (*S*) configuration at C3.

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Experimental Section

General: Melting points (uncorrected): Büchi melting point apparatus. − R_f values: TLC plates Polygram Sil G/UV$_{254}$ (Macherey-Nagel); development: 50% H_2SO_4/120−130°C, 10−15 min. − CC: Silica 32−63 μm (ICN Biomedicals). − Optical rotation: polarimeter 241 (Perkin-Elmer). − NMR spectra: Bruker AC 200 and AVANCE 300 spectrometers (chemical shifts were assigned by C,H-COSY). − MS: I_{eol} IMS-700.

Acetylation and Chromatographic Work-up: 1000 ml of Aronia concentrate[1] was freeze-dried yielding a residue of ca. 150 g. After drying over P_4O_{10}, 15 g of this product was acetylated at room temp. with 30 ml acetic anhydride/25 ml pyridine for 24 h. The reaction mixture was poured into ice/water and triturated until the precipitated oil solidified and could be filtered off (yield 19 g). 10 g of the peracetate mixture was dissolved in acetone and mixed with 20 g of Celite. After removal of the solvent, toluene was added and the mixture was transferred to a column (∅ = 6 cm, length 30 cm, filled with silica gel) and eluted with toluene/acetone (95:5, 9:1, 8:2, and 7:3). The fractions were examined by TLC (toluene/acetone, 8:2). Since the R_f values were very similar, the separate fractions had to be further purified by means of smaller columns (∅ = 4 cm, length 30 cm or ∅ = 2 cm, length 25 cm, depending on the quantity of material). The peracetates crystallized from EtOH. In order to obtain pure compounds, several recrystallizations were necessary and so precise yields cannot be quoted. Products **4** and **7** were found to be the principal constituents and were isolated in quantities of ca. 100 mg.

Hexa-O-acetyl-sorbitol: M.p. 98−99°C (EtOH). The compound was identified by comparison with an authentic sample (chromatography, ^{1}H- and ^{13}C-NMR spectra).

Penta-O-acetylglucopyranose (**1**): M.p. 128−129°C [ref.[11] 131°C (corr.)] − [Since acetylation was carried out in the presence of pyridine, the product was contaminated with a small amount of the α-anomer; detection by ^{1}H NMR: 1-H (α): δ = 6.35, $J_{1/2}$ ca. 4 Hz; 1-H (β): δ = 5.72, $J_{1/2}$ = 8.5 Hz]. − R_f = 0.54 (toluene/acetone, 8:2). Identified by comparison with an authentic sample.

Tetra-O-acetyl-β-D-glucopyranosyl Benzoate (**2**): M.p. 142°C (EtOH). − R_f = 0.55 (toluene/acetone, 8:2). − $[\alpha]_D^{21}$ = −30.2 (*c* = 1.5, acetone). − ^{13}C NMR: see Table 1. The identity was cross-checked by comparison with a sample prepared from tetra-*O*-acetyl-*O*-α-D-glucopyranosyl-trichloroacetimidate[12]. − $C_{21}H_{24}O_{11}$ (452.4): calcd. C 55.75, H 5.35; found C 55.49, H 5.26.

4-O-(Tetra-O-acetyl-β-D-glucopyranosyl)pentan-2-one (**3**): M.p. 125−126°C (EtOH), R_f = 0.39 (toluene/acetone, 8:2). − $[\alpha]_D^{20}$ = −30.6 (*c* = 0.6, acetone). − ^{13}C NMR: See Table 1. − MS (FAB, positive-ion mode): calcd. $[M + Na]^+$ ($C_{19}H_{28}O_{11}Na$) 455.1529; found $[M + Na]^+$ 455.1531. − $C_{19}H_{28}O_{11}$ (432.4): calcd. C 52.77, H 6.53; found C 52.57, H 6.57.

Tetra-O-acetyl Parasorboside (**4**): M.p. 157°C[3] (EtOH), R_f = 0.25 (toluene/acetone, 8:2). − $[\alpha]_D^{20}$ = −17.5 (*c* = 1, acetone). − ^{13}C NMR: See Table 1. − $C_{20}H_{28}O_{12}$ (460.2): calcd. C 52.15, H 6.13; found C 52.06, H 6.28.

Table 3. Crystallographic data

Compound **4**	
Empirical formula	$C_{20}H_{28}O_{12}$
Molecular mass [g/mol]	460.4
Crystal size	0.4 × 0.3 × 0.25
Crystal color	colourless
Crystal shape	plate
Space group	$P2_12_12_1$
a [Å]	9.133(1)
b [Å]	9.952(2)
c [Å]	26.073(5)
V [Å^3]	2369.8(7)
$D_{calcd.}$ [Mg/m^3]	1.29
Z	4
F(000)	976
Temperature [K]	238
h_{min}/h_{max}	0/12
k_{min}/k_{max}	0/13
l_{min}/l_{max}	0/34
$(\sin \Theta/\lambda)_{max}$ [Å^{-1}]	0.66
μ [mm^{-1}]	0.11
Refl. collected	3236
Refl. unique	3236
Refl. observed [$I > 2\ \sigma\ (I)$]	2272
Variables	355
$(\Delta/\sigma)_{max}$	<0.01
R	0.038
R_w	0.084
S (Gof)	1.09
$(\Delta\rho)_{max}$ [eÅ^{-3}]	0.17
$(\Delta\rho)_{min}$ [eÅ^{-3}]	−0.20

Crystal Structure Analysis of **4**: Table 1 contains the crystallographic data and details of the refinement procedure. The reflections were collected with an Enraf-Nonius CAD4 diffractometer (Mo-*Kα* radiation, graphite monochromator, ω−2Θ scan). Intensities were corrected for Lorentz and polarization effects.

The structure was solved by direct methods (SHELXS-86[13]). The structural parameters of the non-hydrogen atoms were refined anisotropically according to a full-matrix least-squares technique (F^2). The parameters of the hydrogen atoms were refined isotropically, except those at the methyl groups, which were calculated. The refinement was carried out with SHELXL-93[14]. One oxygen atom was found to be disordered over two positions with 50% multiplicity (O71′ and O72′). Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-100 590. Copies of the data can be obtained free of charge on application to The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: (internat.) +44 (0)1223/336033; e-mail: deposit@chemcrys.cam.ac.uk].

Hepta-O-acetylamygdalin (**7**): M.p. 167−168°C (EtOH), R_f = 0.35 (toluene/acetone, 8:2), $[\alpha]_D^{20}$ = −38.5 (*c* = 2, $CHCl_3$). ^{13}C NMR: see Table 2. Identified by comparison with an authentic sample.

Hepta-O-acetyl (2-Pentanyl)-β-D-gentiobioside (**8**): M.p. 168°C (EtOH), R_f = 0.42 (toluene/acetone, 8:2); $[\alpha]_D^{20}$ = −21.4 (*c* = 0.7, acetone). ^{13}C NMR: see Table 2. − MS (FAB, positive-ion mode): calcd. $[M + H]^+$ ($C_{31}H_{47}O_{18}$) 707.2762, $[M + Na]^+$ ($C_{31}H_{46}O_{18}Na$) 729.2582; found $[M + H]^+$ 707.2740, $[M + Na]^+$ 729.2560. − $C_{31}H_{46}O_{18}$ (706.7): calcd. C 52.69, H 6.56; found C 52.58, H 6.51.

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