JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Delphinidin 3-*O*-(2-*O*-*β*-D-Glucopyranosyl-α-L-arabinopyranoside): A Novel Anthocyanin Identified in Beluga Black Lentils

GARY R. TAKEOKA,*,† LAN T. DAO,† HIROTOSHI TAMURA,‡ AND LESLIE A. HARDEN†

Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, 800 Buchanan Street, Albany, California 94710, and Department of Biochemistry and Food Science, Kagawa University, 2393 Miki-cho, Kagawa 761-0795, Japan

A major anthocyanin was isolated from the acidified methanolic extract of Beluga black lentils by XAD7 column chromatography and preparative high-performance liquid chromatography. By means of electrospray ionization mass spectrometry and one- and two-dimensional nuclear magnetic resonance spectroscopy, its structure was determined to be delphinidin 3-*O*-(2-*O*-*β*-D-glucopyranosyl-α-L-arabinopyranoside).

KEYWORDS: Anthocyanins; black lentils (*Lens culinaris*); 1D and 2D NMR; delphinidin derivative

INTRODUCTION

Lentils (*Lens culinaris*) are small annual legumes of the pea family (Leguminosae). They are one of the oldest crops cultivated by humans, domesticated around 8000 BC in the Fertile Crescent (*1*). The lens-shaped edible seeds are mainly used in soups and are a good source of protein, dietary fiber, folate, iron, and phosphorus. Flour made from lentils is gluten free and may be added to cereal flour to make breads, cakes, and baby foods. The seed coat color can be clear, green, tan, gray, brown, or black while the cotyledon is yellow, red, or green. The main market types are red and green, which together account for an estimated 95% of the world's lentil production (*2*). U.S. lentil production, occurring in the states of Washington, Idaho, Montana, and North Dakota, totaled about 234 million lbs. in 2002−2003 (*2*), mainly with green and brown types. Because of the possible health benefits of anthocyanins (*3*), we decided to investigate their presence in the less utilized Beluga black lentil.

The subepidermal pigment in lentil stem was reported to have delphinidin as the aglycon (*4*). Lentil seeds were reported to contain a compound that resembled a diglycosyl derivative of delphinidin (*5*). Because of its low concentration (about 60 ppm), the authors were unable to fully characterize its structure. Slinkard and Bhatty (*6*) reported that stems of seedlings and plants of the large-seeded (Chilean type) lentil variety, Laird, are pigmented with anthocyanins. Anthocyanins are located essentially in their seed coat, which constitutes about 8−11% of the entire lentil seed (*7*). The goal of this work was to elucidate the structure of the major anthocyanin(s) in Beluga black lentils.

EXPERIMENTAL PROCEDURES

Materials. Beluga black lentils were obtained from George F. Brocke and Sons, Inc. (Kendrick, ID). Concord grape puree was obtained from the Milne Fruit Products, Inc. (Prosser, WA). Methyl *β*-L-arabinopyranoside was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Solvents were of high-performance liquid chromatography (HPLC) spectroquality grade unless otherwise stated.

Extraction Method. Lentils (1.26 kg) were ground to a powder with a UDY Cyclone sample mill (Fort Collins, CO). The powder was mixed with methanol containing 3% HCl (2.8 L), and the resulting mixture was allowed to stand overnight in a refrigerator. The mixture was filtered through a Whatman #4 filter (24.0 cm) using a Buchner funnel. The powder was mixed with methanol containing 0.3% HCl (1.8 L). The mixture was allowed to stand overnight in a refrigerator and was then filtered as above. The combined extract was concentrated to ca. 100 mL with a rotary evaporator. Trifluoroacetic acid (TFA), a weaker acid than HCl, is preferred for the extraction of anthocyanins since it is less likely to cause pigment degradation, particularly during the concentration step (*8*). We did notice anthocyanin degradation, especially during concentration of the methanol extract containing 3% HCl. Subsequent extractions were done using TFA as the acid.

Amberlite XAD7 Column Chromatography. Amberlite XAD7 resin (600 mL; 20−60 mesh [wet]; average diameter, 90 Å; pore volume, 1.14 mL/g) was conditioned in the following way: The resin was mixed with methanol (2 L), and the upper layer was decanted to remove the white colored solution. This procedure was repeated with 1 L of methanol. The resin was packed into a glass column (4.0 cm i.d. × 80 cm; resin volume, 1.0 L) and eluted with 4 L of acetone, 1 L of methanol, 1 L of methanol containing 0.3% HCl, 1 L of 50:50 (v/v) methanol:water containing 0.3% HCl, and 1.5 L of 0.3% HCl. The anthocyanin extract was diluted two times in purified water and applied to the column. The column was eluted with 3 L of 0.3% HCl to remove polar constituents. The column was subsequently eluted in

* To whom correspondence should be addressed. Tel: 510-559-5668. Fax: 510-559-5828. E-mail: grt@pw.usda.gov.
† U.S. Department of Agriculture.
‡ Kagawa University.

10.1021/jf040493h CCC: $30.25
Published on Web 05/12/2005

a stepwise fashion with 10:90 (v/v) methanol:water containing 0.3% HCl, 20:80 (v/v) methanol:water containing 0.3% HCl, 30:70 (v/v) methanol:water containing 0.3% HCl, 40:60 (v/v) methanol:water containing 0.3% HCl, 50:50 (v/v) methanol:water containing 1% TFA, and 60:40 (v/v) methanol:water containing 1% TFA.

Thin-Layer Chromatography (TLC). Precoated RP-18 F_{254}s TLC plates (Merck; layer thickness, 0.25 mm) were used with a solvent system of acetic acid:acetonitrile:phosphoric acid:water (10:12.5:1.5:76) to evaluate the XAD7 column fractions.

Analytical HPLC. A Hewlett-Packard 1100 Series HPLC system consisting of an 1100 Series quaternary pump, an 1100 Series vacuum degasser, a manual injector (model 7725i, Rheodyne, Rohnert Park, CA), equipped with a 20 μL sample loop, an 1100 Series thermostated column compartment, and an 1100 Series diode array detector was used. The instrument was controlled and data were processed by an HP ChemStation for LC 3D (Rev. A.08.03 [847]). The analytical column was an ODS/B (250 mm × 4.6 mm i.d., 5 μm, 100 Å; Keystone Scientific Inc., Bellefonte, PA) protected by a Supelguard LC-18-DB (Supelco, Inc., Bellefonte, PA) guard column. Solvent A consisted of 10% aqueous formic acid, and solvent B was formic acid/water/methanol (10:40:50 v/v). Separations were performed with a linear gradient of 40−80% solvent B over a period of 50 min at a flow rate of 1.0 mL/min and detection at 525 nm. Sample volumes of 50 μL were used for injection.

Preparative HPLC. The preparative HPLC system consisted of Gilson model 305 and 306 pumps, a Gilson 806 manometric module, a Gilson 811C dynamic mixer (Gilson Medical Electronics, Middleton, WI), a manual injector (Rheodyne model 7125) fitted with a 200 μL sample loop, and a Gilson model 112 UV detector equipped with a preparative flow cell. A C18 reversed phase Dynamax preparative column (250 mm × 21.4 mm i.d., 8 μm, 100 Å; Varian, Inc., Walnut Creek, CA) was coupled directly to a guard column (50 mm × 21.4 mm i.d.) containing the same packing material. The mobile phase was the same as used for analytical HPLC. Separations were performed with a linear gradient of 50−80% solvent B over a period of 15 min at a flow rate of 8 mL/min. The detector was set at 525 nm. The major anthocyanin was isolated, and its purity was checked by analytical HPLC.

Acid Hydrolysis of Anthocyanins. Concord grape puree and the purified lentil anthocyanin were evaporated to near dryness by rotary evaporation and then redissolved in 10% aqueous formic acid. Two milliliters of the sample was heated with 2 mL of 2 N HCl in a boiling water bath for 60 min and then cooled in an ice bath. Shorter heating times were also used to do controlled acid hydrolysis (*8*). The hydrolysate was adsorbed on a C18 Bond Elut cartridge (3 cm^3/500 mg sorbent; Varian, Inc.) previously conditioned with methanol followed by water (*9*). The sugar fraction was eluted from the cartridge with 0.1% HCl, concentrated to dryness, and redissolved in ethanol/water (50:50 v/v). The anthocyanidins remaining in the cartridge were eluted with 0.1% HCl/MeOH and analyzed by HPLC promptly due to the instability of anthocyanidins (*10*, *11*).

Identification of Carbohydrates by TLC. Fifteen microliters of the sugar fraction was spotted on a Whatman K5 silica gel (Alltech Associates, Deerfield, IL) TLC plate (20 cm × 20 cm, activated for 60 min at 105 °C) along with carbohydrate standards. After development with 2-propanol/acetone/0.1 M lactic acid (4:4:2 v/v), the plate was air-dried and the sugars were visualized by spraying with aniline/diphenylamine/acetone/80% phosphoric acid (4 mL:4 g:200 mL:30 mL) and heating for 30 min at 105 °C (*12*).

Identification of Anthocyanidins. Because of the limited availability of anthocyanidin standards, concord grape puree was used as a source of five of the most common anthocyanidins with the exception of pelargonidin (*13*). The HPLC elution order is as follows: dephinidin, cyanidin, petunidin, pelargonidin, peonidin, and malvidin (*14*, *15*).

Preparation of Methyloxime-Trimethylsilyl (MO-TMS) Derivatives of Carbohydrates for Gas Chromatography (GC) and GC/MS Analyses. Carbohydrate samples were isolated using C18 SPE cartridges using the same procedure as for analyses of sugars by TLC. A mixture of dry pyridine (Mallinckrodt, St. Louis, MO) and 1 mg of *O*-methylhydroxylamine-HCl (TCI America, Portland, OR) was added to 1 mg of each carbohydrate sample and standard. Carbohydrate

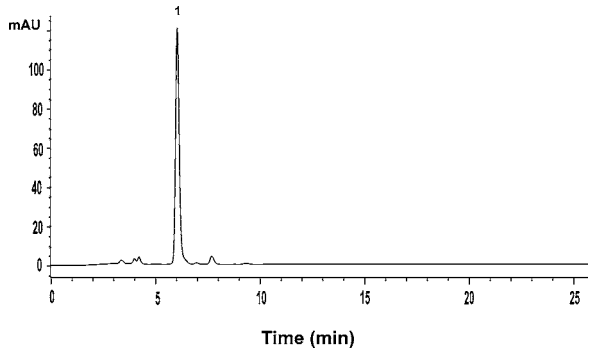

Figure 1. HPLC chromatogram (detection at 525 nm) of Beluga black lentil anthocyanins. Conditions: column, Keystone ODS/B 250 mm × 4.6 mm i.d.; solvent A, 10% formic acid; solvent B, formic acid/methanol/water (10:40:50 v/v); linear gradient from 40 to 80% solvent B in 50 min. Peak 1, delphinidin 3-*O*-(2-*O*-β-D-glucopyranosyl-α-L-arabinopyranoside).

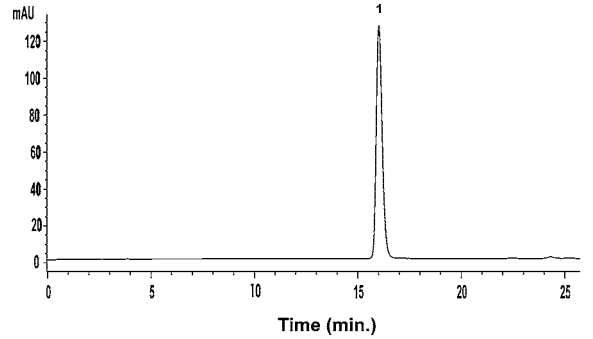

Figure 2. HPLC chromatogram (detection at 525 nm) of Beluga black lentil anthocyanidin (aglycon). Conditions were as in Figure 1. Peak 1, delphinidin.

standards were obtained from Sigma Chemical Co. (St. Louis, MO). The solutions were mixed well, heated for 2 h at 40 °C, and allowed to stand overnight at room temperature. Pyridine was removed in a stream of nitrogen while the sample was heated to 40 °C. When the solution approached dryness, two drops of benzene was added to azeotropically remove the last trace of water formed in the oximation reaction. One hundred microliters of a 99:1 mixture of N,O-bis-(trimethyl)trifluoroacetamide plus trimethylchlorosilane (Sylon BFT, Supelco, Inc.) was added to each sample. The mixtures were shaken well, heated for 2 h at 40 °C, and allowed to stand overnight at room temperature. This procedure is a modification of the method of Laine and Sweeley (*16*, *17*) for the preparation of MO-TMS derivatives of aldoses, partially methylated aldoses, deoxyaldoses, and ketoses.

Capillary GC. An HP 5890 Series II gas chromatograph equipped with a flame ionization detector was used. A DB-1 fused silica capillary column (60 m × 0.32 mm i.d.; d_f = 0.25 μm; J&W Scientific, Folsom, CA) was employed. The linear velocity of the helium carrier gas was 35 cm/s (30 °C). Split injection was used (1:25). The oven temperature was programmed from 30 (4 min isothermal) to 110 °C at 8 C/min and then to 200 °C at 2 °C/min (final time, 35 min). The injector and detector temperatures were 190 and 290 °C, respectively. The instrument was controlled and data were processed by an HP ChemStation (Rev. A.06.01 [403]).

Capillary GC/MS. An HP 6890 Plus gas chromatograph equipped with a split/splitless injector was coupled to an HP 5973 mass selective detector. The instrument was controlled and data were processed by an HP Enhanced ChemStation (G1701CA version C.00.00 21 Dec. 1999). A 60 m × 0.25 mm i.d. (d_f = 0.25 μm) DB-1 fused silica capillary column was used with the following temperature program: 30 (4 min isothermal) to 110 °C at 8 C/min and then to 200 °C at 2 °C/min (final time, 40 min). Helium carrier gas was employed at a column headpressure of 22 psi. The injector temperature was 190 °C, and the transfer line temperature was 200 °C. The ion source and quadrupole temperatures were 170 and 130 °C, respectively.

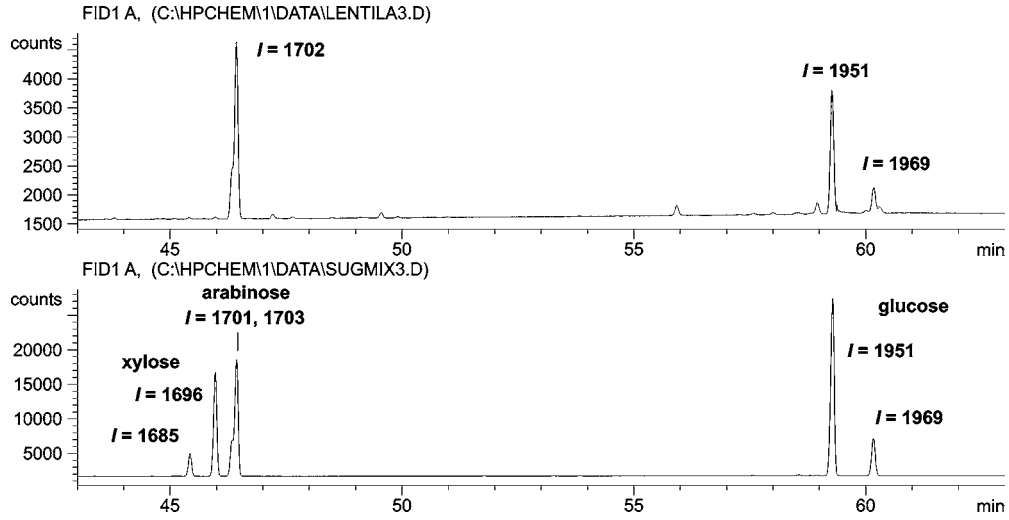

Figure 3. Gas chromatograms of the MO-TMS derivatives of the sugars released by hydrolysis of the major lentil anthocyanin (top) and D-xylose, L-arabinose, and D-glucose standards (bottom). The numbers (*I*) represent Kováts indices. The conditions are described in the Experimental Procedures.

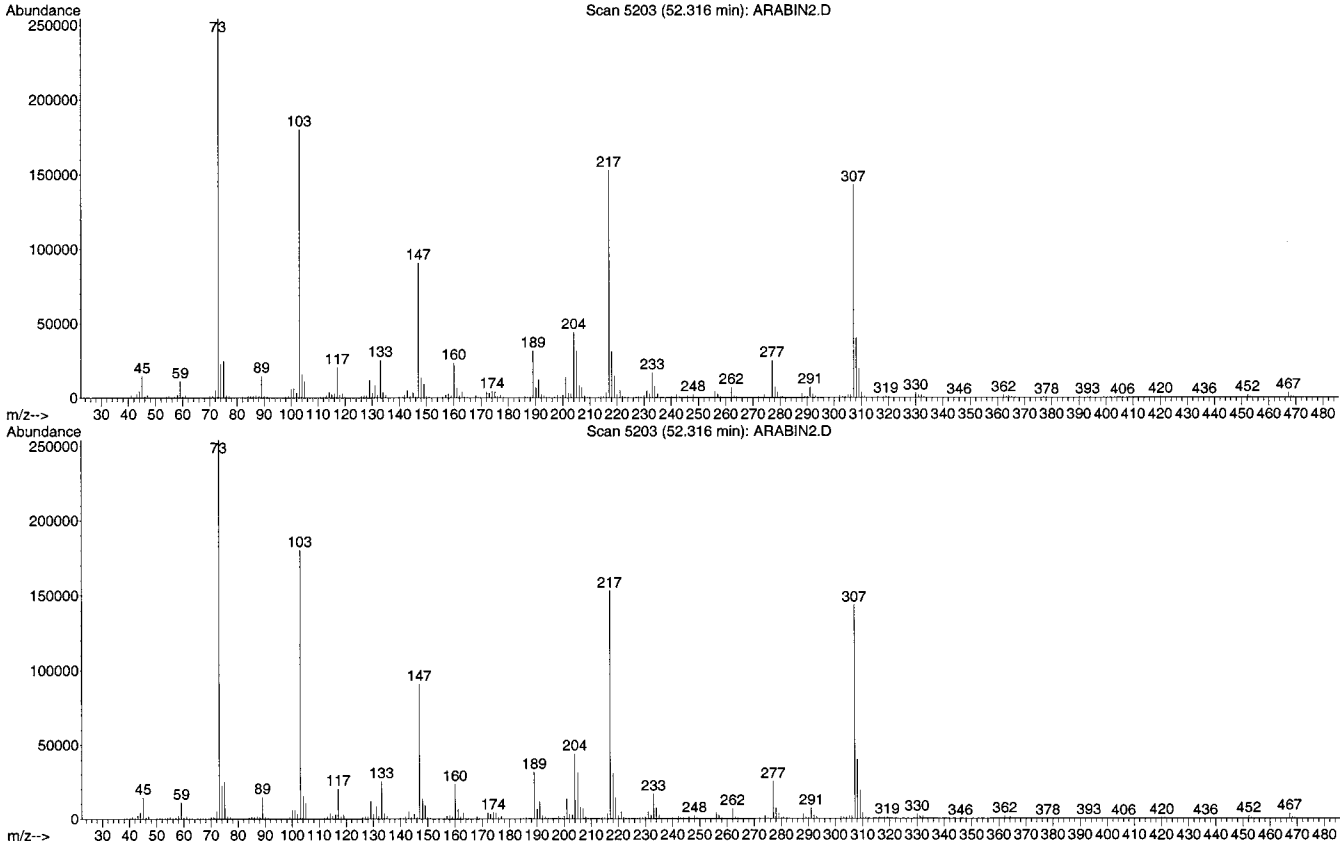

Figure 4. Mass spectra of the MO-TMS derivatives of one of the sugars released by hydrolysis of the major anthocyanin (top) and the L-arabinose standard (bottom). The conditions are described in the Experimental Procedures.

Electrospray Ionization MS/MS. A hybrid quadrupole time-of-flight mass spectrometer, API QSTAR Pulsar (Applied Biosystems/PE Sciex, Foster City, CA) equipped with a nanoelectrospray ion source (Protana Engineering, now known as Proxeon Biosystems, Odense M, Denmark) was used. The sample was diluted in 50:50 (v/v) methanol:water with 0.1% TFA and sprayed with a nanospray emitter (Proxeon Biosystems). The instrument was operated in positive ion mode with a spray voltage of 1100 V. The collision gas was nitrogen, and the collision energy was 20 eV. The scan range for product ions in TOF was 100.0000−1000.0000 *m/z*.

NMR Spectroscopy. ^{1}H NMR spectra of the anthocyanin in TFA-d_1:DMSO-d_6 (10:90) at 40 °C and TFA:methanol-d_4 (5:95) at 23 °C were recorded on a JEOL-400 NMR spectrometer (400 MHz, JEOL, Tokyo, Japan) using tetramethylsilane as an internal reference whereas ^{13}C NMR spectra were run at 100 MHz.

RESULTS AND DISCUSSION

The major anthocyanin was eluted from the XAD7 column with the solvent mixture of 50:50 (v/v) methanol:water containing 1% TFA. The partially purified anthocyanin (120 mg) was subjected to further cleanup using preparative HPLC.

HPLC Separation of Black Lentil Anthocyanins and Anthocyanidins. HPLC of black lentil extract revealed one major anthocyanin with a retention time of 6.1 min (**Figure 1**).

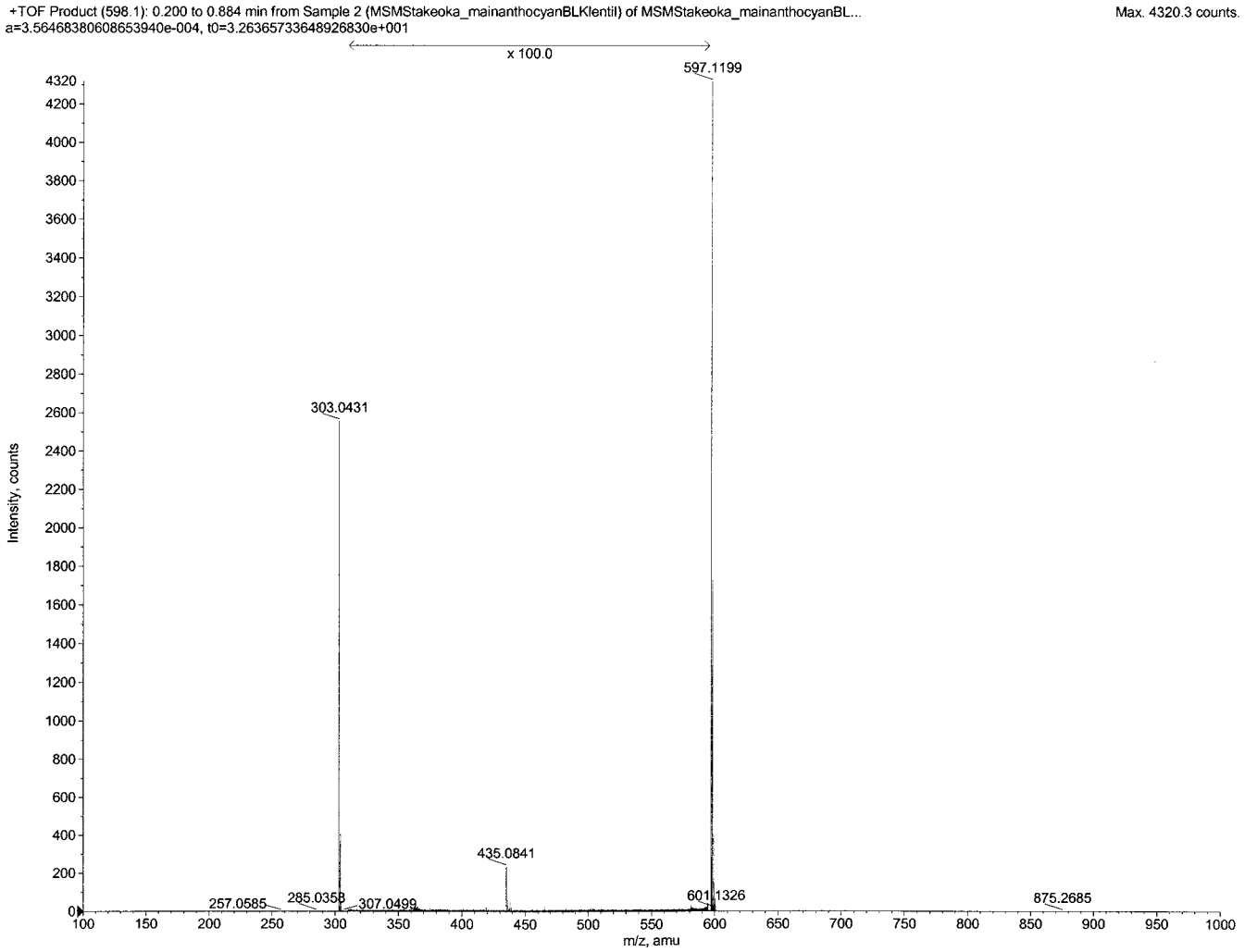

Figure 5. Positive ion ESI-MS/MS product ion spectrum of the major lentil anthocyanin. The MS/MS spectrum was obtained by isolating and fragmenting the molecular ion [M^+] at *m*/*z* 597.1199. The ion at *m*/*z* 303.0431 corresponds to the aglycon delphinidin.

The UV−vis on-line spectrum using a diode array HPLC detector showed that the anthocyanin had its λ_{max} in the 525 nm region. The anthocyanin was not acylated with aromatic acids as evidenced by the absence of peaks in the 300−350 nm region (*18*). Acid hydrolysis of the purified lentil anthocyanin showed one anthocyanidin with a retention time of 16.1 min (**Figure 2**). Tentative identification of the anthocyanidin was achieved by analyzing anthocyanidins obtained from natural sources. The anthocyanidin from lentil had a retention time consistent with that of delphinidin.

Identification of Arabinose and Glucose from the Acid Hydrolysis of a Black Lentil Anthocyanin. The major anthocyanin was isolated by preparative HPLC and subjected to acid hydrolysis to liberate an anthocyanidin and carbohydrates. The carbohydrates were isolated from the other hydrolysis products by SPE. TLC of carbohydrate samples and standards showed one blue spot with an R_f value of 0.45, identical with that of the D-glucose standard. Another spot (purple color) with an R_f value of 0.53 could not be identified with certainty since the L-arabinose and D-xylose standards had nearly identical R_f values. The carbohydrates in the hydrolysate were converted to MO-TMS derivatives and analyzed by capillary GC and GC/MS. The GC chromatogram showed two major peaks with Kováts indices of 1702 and 1951, which had identical retention indices as derivatized L-arabinose and D-glucose, respectively (**Figure 3**). The first peak had a shoulder on its leading edge that had a nearly identical mass spectrum as the main peak. The unresolved peaks are believed to be the syn and anti isomers of the *O*-methyloxime (*16*, *17*) of L-arabinose. There was a minor peak with a Kováts index of 1969 that eluted after the second major peak (I = 1951). These two peaks had identical retention indices and peak area ratios as derivatized D-glucose standard. The two peaks that had nearly identical mass spectra were again believed to be the syn and anti isomers of the *O*-methyloxime of D-glucose. The mass spectra of the MO-TMS derivatives of the carbohydrates liberated from the isolated anthocyanin closely matched those of the L-arabinose (**Figure 4**) and D-glucose standards.

MS/MS. The purified anthocyanin was diluted to a suitable volume in 50:50 (v/v) methanol:water with 0.1% TFA. The electrospray mass spectrum of the purified anthocyanin exhibited a molecular ion [M^+] as the flavylium cation at *m*/*z* 597.1199 (the exact mass calculated for $C_{26}H_{29}O_{16}$ was *m*/*z* 597.1456). MS/MS of the molecular ion gave an ion at *m*/*z* 303.0431 corresponding to the aglycon delphinidin (the exact mass calculated for $[C_{15}H_{11}O_7]^+$ was *m*/*z* 303.0505), which was formed by loss of arabinose and glucose. One 100-fold magnification of the region between 303 and 597 revealed a small ion at *m*/*z* 435.0841 (the exact mass calculated for $C_{20}H_{19}O_{11}$ was *m*/*z* 435.0927), indicating loss of glucose (162, glucose − H_2O) (**Figure 5**). This showed that arabinose was connected directly to delphinidin.

NMR Spectroscopy. NMR spectroscopy was employed to elucidate the positions of attachments of the sugars as well as

Table 1. ^{1}H and ^{13}C NMR Data for Delphinidin 3-*O*-(2-*O*-β-D-Glucopyranosyl-α-L-arabinopyranoside) in 10% TFA–DMSO at 40 °C

	^{1}H NMR (ppm)		^{13}C NMR (ppm)
		delphinidin	
4	8.67	s	132.9
6	6.71	bs	102.5
8	6.82	bs	94.0
2′	7.79	s	111.8
6′	7.79	s	111.8
		O-α-arabinopyranoside	
1	5.59	d (J = 4.2 Hz)	99.28
2	4.18	t (J = 5.4 Hz)	78.80
3	3.85	m	69.72
4	3.85	m	65.07
5a	3.75	dd (J = 5.6, 11.0 Hz)	63.35
5b	3.59	dd (J = 2.2, 11.0 Hz)	63.35
		O-β-glucopyranosyl	
1	4.42	d (J = 7.8 Hz)	104.17
2	2.93	t (J = 8.5 Hz)	74.02
3	3.09	t (J = 9.0 Hz)	76.59
4	3.01	t (J = 8.8 Hz)	69.42
5	2.83	m	76.28
6a	3.20	dd (J = 4.4, 11.5 Hz)	60.31
6b	3.15	dd (J = 2.2, 11.5 Hz)	60.31

their conformations. The presence of delphinidin was confirmed by the observation of one signal belonging to the B-ring protons at 7.79 ppm (**Table 1**). The signals at C-4, C-6, and C-8 were in accord with our previous studies of delphindin 3-glucoside (*19*). A characteristic coupling constant of 2 Hz was observed for the meta coupling between the H-6 and the H-8 protons (observed with 5% TFA in CD_3OD at 23 °C). The glycosidic protons were found as doublets downfield of the other sugar protons in the region from 4.4 to 5.6 ppm. The attachment of the arabinose to the C-3 position of delphinidin was indicated by the nuclear Overhauser effect (NOE) observed with H-1″ of arabinose and H-4 of delphinidin. For pyranoses, a coupling constant of 7.5 Hz indicates a trans dihedral angle for H-1 and H-2, a β-linkage, while a coupling constant of less than 3 Hz indicates a gauche dihedral angle, an α-linkage. The H-1‴ of glucose had a coupling constant of 7.8 Hz, which indicated the β-form. ^{1}H coupling constants of the other three proton signals on the same sugar had values greater than 8.5 Hz ($J_{H\text{-}2'''}$ = 8.5, $J_{H\text{-}3'''}$ = 9.0, and $J_{H\text{-}4'''}$ = 8.8), indicating the presence of the glucopyranose form (evidence of all trans configuration in those proton signals). The carbon signals were similar to those of methyl-D-glucopyranoside (*20*). The position of attachment between glucose and arabinose was established by observation of an NOE between H-2″ of arabinose with H-1‴ of glucose. The remaining proton and carbon signals are consistent with that of an *O*-α-L-arabinopyranoside (*21*−*23*) with the exception of C-2″, which was shifted significantly downfield due to the glycosylation at C-2″. An HMBC spectrum found correlation between H-2″ and C-3″ and between H-5″ and C-4″. As opposed to glucose, which occurs in the D-form, arabinose occurs in the L-form. With L-arabinose, α-linkages should exhibit larger coupling constants (because of axial−axial coupling between protons) than β-linkages. An intermediate coupling constant of 4.2 Hz was observed with a solvent of 10:90 TFA:DMSO at 40 °C while a higher coupling constant of 5.1 Hz was seen with a solvent of 5:95 TFA:methanol at 23 °C. The values of 4.2 and 5.1 Hz for the anomeric proton were consistent with an α-linkage. A coupling constant of 5.1 Hz was reported for the anomeric proton of quercetin 3-*O*-α-L-arabinopyranoside using DMSO as the solvent (*21*). In addition,

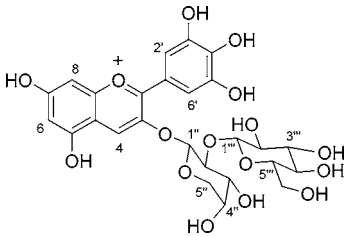

Figure 6. Structure of delphinidin 3-*O*-(2-*O*-β-D-glucopyranosyl-α-L-arabinopyranoside).

we found a smaller coupling constant of 2 Hz for the anomeric proton of methyl β-L-arabinopyranoside using a solvent of 10:90 TFA:DMSO at 40 °C. On the basis of the NMR data, the major anthocyanin was determined to be delphinidin 3-*O*-(2-*O*-β-D-glucopyranosyl-α-L-arabinopyranoside) (**Figure 6**).

LITERATURE CITED

(1) Vaughan, J. G.; Geissler, C. *The New Oxford Book of Food Plants*; Oxford University Press Inc.: New York, 1997; pp 46−47.

(2) Agriculture and Agri-Food Canada Bi-weekly bulletin. Lentils/fababeans. **2004**, *17* (8), 1−4.

(3) Kong, J.-M.; Chia, L.-S.; Goh, N.-K.; Chia, T.-F.; Brouillard, R. Analysis and biological activities of anthocyanins. *Phytochemistry* **2003**, *64*, 923−933.

(4) Nozzolillo, C. A survey of anthocyanin pigments in seedling legumes. *Can. J. Bot.* **1973**, *51*, 911−915.

(5) D'Arcy, A.; Jay, M. Les flavanoides des graines de *Lens culinaris*. *Phytochemistry* **1978**, *17*, 826−827.

(6) Slinkard, A. E.; Bhatty, R. S. Laird lentil. *Can. J. Plant Sci.* **1979**, *59*, 503−504.

(7) Dueas, M.; Sun, B.; Hernndez, T.; Estrella, I.; Spranger, M. I. Proanthocyanidin composition in the seed coat of lentils (*Lens culinaris* L.). *J. Agric. Food Chem.* **2003**, *51*, 7999−8004.

(8) Strack, D.; Wray, V. Anthocyanins. In *Methods in Plant Biochemistry*; Harborne, J. B., Ed.; Academic Press: London, 1989; Vol. 1, pp 325−356.

(9) Dao, L. T.; Takeoka, G. R.; Edwards, R. H.; Berrios, J. De J. Improved method for the stabilization of anthocyanidins. *J. Agric. Food Chem.* **1998**, *46*, 3564−3569.

(10) Harborne, J. B. *Comparative Biochemistry of the Flavonoids*; Academic Press: London, 1967.

(11) Iacobucci, G. A.; Sweeny, J. G. The chemistry of anthocyanins, anthocyanidins and related flavylium salts. *Tetrahedron* **1983**, *39*, 3005−3038.

(12) Hansen, S. A. Thin-layer chromatographic method for identification of mono-, di-, and trisaccharides. *J. Chromatogr.* **1975**, *107*, 224−226.

(13) Hong, V.; Wrolstad, R. E. Cranberry juice composition. *J. Assoc. Off. Anal. Chem.* **1986**, *69*, 199−207.

(14) Karppa, J.; Kallio, H.; Peltonen, I.; Linko, R. Anthocyanins of crowberry, *Empetrum nigrum* coll. *J. Food Sci.* **1984**, *49*, 634−636.

(15) Wilkinson, M.; Sweeny, J. G.; Iacobucci, G. A. High-pressure liquid chromatography of anthocyanins. *J. Chromatogr.* **1977**, *132*, 349−351.

(16) Laine, R. A.; Sweeley, C. C. Analysis of trimethylsilyl *O*-methyloximes of carbohydrates by combined gas−liquid chromatography−mass spectrometry. *Anal. Biochem.* **1971**, *43*, 533−538.

(17) Laine, R. A.; Sweeley, C. C. *O*-methyl oximes of sugars. Analysis as *O*-trimethylsilyl derivatives by gas−liquid chromatography and mass spectrometry. *Carbohydr. Res.* **1973**, *27*, 199−213.

(18) Andersen, M. Chromatographic separation of anthocyanins in Cowberry (Lingonberry) *Vaccinium vites-Idaea* L. *J. Food Sci.* **1985**, *50*, 1230−1232.

(19) Takeoka, G. R.; Dao, L. T.; Full, G. H.; Wong, R. Y.; Harden, L. A.; Edwards, R. H.; Berrios, J. De J. Characterization of black bean (*Phaseolus vulgaris* L.) anthocyanins. *J. Agric. Food Chem.* **1997**, *45*, 3395−3400.

(20) Agrawal, P. NMR spectroscopy in the structural elucidation of oligosaccharides and glycosides. *Phytochemistry* **1992**, *31*, 3307−3330.

(21) Fraisse, D.; Heitz, A.; Carnat, A.; Carnat, A.-P.; Lamaison, J.-L. Quercetin 3-arabinopyranoside, a major flavonoid compound from *Alchemilla xanthochlora*. *Fitoterapia* **2000**, *71*, 463−464.

(22) Markham, K. R.; Ternai, B.; Stanley, R.; Geiger, H.; Mabry, T. J. Carbon-13 NMR studies of flavonoids. III. Naturally occurring flavonoid glycosides and their acylated derivatives. *Tetrahedron* **1978**, *34*, 1389−1397.

(23) Pachaly, P.; Klein, M. Inhaltsstoffe von *Andromeda polifolia*. *Planta Med*. **1987**, *53*, 442−444.

Received for review December 23, 2004. Revised manuscript received April 4, 2005. Accepted April 7, 2005.

JF040493H