New Highly Stable Dimeric 3-Deoxyanthocyanidin Pigments from *Sorghum bicolor* Leaf Sheath

Bhimalingeswarappa Geera, Leonnard O. Ojwang, and Joseph M. Awika

Abstract: The growing interest in natural alternatives to synthetic petroleum-based dyes for food applications necessitates looking at nontraditional sources of natural colors. Certain sorghum varieties accumulate large amounts of poorly characterized pigments in their nongrain tissue. We used High Performance Liquid Chromatography-Tandem Mass Spectroscopy to characterize sorghum leaf sheath pigments and measured the stability of isolated pigments in the presence of bisulfite at pH 1.0 to 7.0 over a 4-wk period. Two new 3-deoxyanthocyanidin compounds were identified: apigeninidin-flavene dimer and apigenin-7-O-methylflavene dimer. The dimeric molecules had near identical UV-Vis absorbance profiles at pH 1.0 to 7.0, with no obvious sign of chalcone or quinoidal base formation even at the neutral pH, indicating unusually strong resistance to hydrophilic attack. The dimeric 3-deoxyanthocyanidins were also highly resistant to nucleophilic attack by SO_2; for example, apigeninidin-flavene dimer lost less than 20% of absorbance, compared to apigeninidin monomer, which lost more than 80% of absorbance at λ_{max} within 1 h in the presence of SO_2. The increased molecular complexity of the dimeric 3-deoxyanthocyanidins compared to their monomers may be responsible for their unusual stability in the presence of bisulfite; these compounds present new interesting opportunities for food applications.

Keywords: anthocyanin, color stability, 3-deoxyanthocyanin, pigments, sorghum

Practical Application: Natural dimeric pigments concentrated in sorghum sheath retain their hue properties and stability over a wide pH range and should be explored for potential use in mildly acidic to neutral food applications.

Introduction

The food industry is increasingly searching for natural colorants to replace the synthetic petroleum-based dyes commonly used in foods. The past few years has seen significant growth in the natural colorants market. Many factors have contributed to this, including tightening government regulations, and a growing consumer desire for clean food labels. Recent evidence linking attention deficit hyperactivity disorder (ADHD) in children to consumption of the synthetic dyes (Bateman and others 2004), and the subsequent press coverage have only added to consumer anxiety about synthetic food additives. In 2010, food products containing artificial dyes marketed in the European Union require a warning label on the possible link to ADHD in children (UKFSA 2010). Food colorants play an important role in enhancing the aesthetic appeal of food, and finding natural colorants that provide the same functionality (hue, stability, and so on) as the artificial dyes at reasonable cost remains a challenge.

Sorghum accumulates large quantities of the rare 3-deoxyanthocyanin pigments. The sorghum pigments, with their red-orange hues in mildly acidic to neutral solutions, and better stability compared to anthocyanins (Mazza and Brouillard 1987; Awika and others 2004) have gained interest as potential natural food colorants to complement available options. However, hurdles exist in commercially exploiting these interesting pigments, particularly the fact that they are relatively difficult to extract with aqueous solvents from the bran portion of grain where they are concentrated. Additionally sorghum grain contains mostly the simple 3-deoxyanthocyanidin aglycones, luteolinidin and apigeninidin (Awika and others 2005); though more stable than anthocyanins, these compounds are still susceptible to the bleaching effect of food additives like ascorbic acid and bisulfites (Ojwang and Awika 2008; Ojwang and Awika 2010). Thus to make these pigments commercially competitive, their extractability and stability in the presence of common bleaching agents like ascorbic acid and SO_2 should be addressed.

Recent reports indicate that certain West African sorghum varieties synthesize exceptionally high levels of 3-deoxyanthocyanin pigments in their nongrain tissue (Kayodé and others 2011). An unusual fused-ring apigeninidin derivative (pyrano-apigeninidin-4-vinylphenol) was recently reported in sorghum leaf sheath (Khalil and others 2010). Such compounds have not been identified in sorghum grain, suggesting nongrain tissue may accumulate a greater diversity of more complex 3-deoxyanthocyanin pigments than found in grain. The complex forms of 3-deoxyanthocyanins are likely to be more stable, based on observed improved stability of complexed anthocyanins (Rodriguez-Saona and others 1999; Giusti and Wrolstad 2003). Compared to sorghum bran, the sheaths and glumes contribute a much greater proportion of plant biomass and may provide a more cost-effective way to obtain large quantities of the relatively stable 3-deoxyanthocyanin

MS 20111407 Submitted 11/21/2011, Accepted 2/2/2012. *Authors Geera, Ojwang, and Awika are with Soil & Crop Science Dept., Texas A&M Univ., College Station, TX 77843-2474, U.S.A. Author Geera is currently with J.R. Short Milling Co., Kankakee, IL 60901, U.S.A. Direct inquiries to author Awika (E-mail: jawika@ag.tamu.edu).*

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pigments. In this work, we report previously unidentified dimeric 3-deoxyanthocyanidin pigments in sorghum leaf sheath and investigate their stability to pH change and presence of bisulfite.

Materials and Methods

Dried intensely colored *Sorghum bicolor* leaf sheath powder was provided by Health Forever Products (Lagos, Nigeria). The source of this material is identical to the West African dye sorghum materials recently described by Kayodé and others (2011). Apigeninidin and 7-O-methylapigeninidin standards were purchased from Alsachim (Strasburg, France).

Sample extraction

The sorghum leaf sheath powder was suspended in 50% aqueous methanol and extracted for 30 min, with shaking at room temperature. Extracts were centrifuged (7500 × *g*, 10 min) and supernatants rotoevaporated to remove methanol and then freeze-dried. The freeze-dried extract was dissolved in 20% aqueous methanol and partitioned with equal volume of ethyl acetate. The ethyl acetate fraction was concentrated in vacuo and stored under refrigerated conditions.

HPLC analysis

HPLC separation and purification was achieved using an Agilent 1200 system, equipped with a diode array detector (DAD), quaternary pump, and fraction collector. A Kinetex C18 75 × 4.6 mm i.d., 2.6 micron column (Phenomenex, Torrance, Calif., U.S.A.) thermostated at 40 °C was used. Flow rate was 1.0 mL/min; spectra were recorded from 200 to 700 nm and the monitoring wavelength was 480 nm. The mobile phase consisted of (A), 2% formic acid in water, and (B), 2% formic acid in 50:50 acetonitrile/methanol. The elution gradient for B was as follows: 0 to 3 min, 10% isocratic; 3 to 8 min, 10% to 20% B; 8 to 17 min, 20% to 40% B; 17 to 23 min, 40% to 50% B; 23 to 27 min, 50% B isocratic; 27 to 30 min, 10% B. Fraction collection (for mass spectroscopy analysis) was peak based with a minimum gradient set to 20.

Mass spectroscopy analysis

The isolated HPLC fractions were analyzed using an MDS Sciex API QStar Pulsar mass spectrometer with electrospray ionization (AB SCIEX, Foster City, Calif., U.S.A.); this is a hybrid quadrupole/time-of-flight instrument. Test conditions were as follows: Ions source gas, 40 arbitrary units (au); curtain gas flow rate, 20 au; gas used, nitrogen; ion spray voltage, 4500 V; detector (MCP), 2150 V; syringe pump flow, 6 μL/min. Monomeric 3-deoxyanthocyanidin identification was based on matching HPLC retention profile, UV-Vis spectra, and MS data with authentic standards. Unknown compounds were fragmented at 33% relative collision energy, sheath gas flow rate of 20 au, and auxiliary gas flow rate of 10 au, to further aid in structure determination.

Semipreparative purification

To purify the identified dimeric molecules for color stability assay, the freeze-dried crude extracts were dissolved in 50% methanol adjusted to pH 2.0 with formic acid, filtered through a 0.45 μm membrane, and directly fractionated on a semipreparative Luna 250 × 10 mm C-18(2) column (Phenomenex) with the aid of the Agilent system described earlier. The system was modified by attaching a 1400 μL injection loop to the autosampler, and replacing the standard flow cell with a micro flow cell (3 mm path length) in the detector. Separation conditions were as follows: flow rate, 5 mL/min; injection volume, 300 μL; autosampler temperature, 4 °C; column temperature, 40 °C; monitoring wavelengths, 480 nm. Solvents were as described for analytical HPLC and gradient was as previously reported (Yang and others 2009). The procedure was repeated and individual fractions were pooled until enough of the desired compounds were obtained for analysis. Fraction purity was checked by injecting the pooled fractions and using the analytical HPLC procedure described earlier; separation was effective, and all fractions were >95% pure. The pooled fractions were freeze-dried and kept at –35 °C until used. The content of the purified compounds in the original material was determined by interpolation, based on peak areas from HPLC standard curves for each compound.

Color stability test

Two compounds were selected for this assay to help understand the effect of dimerization on 3-deoxyanthocyanidins to pH change and nucleophilic attack; apigeninidin and its dimer. This was based on the fact that preliminary investigations (data not shown) revealed that the 2 major dimers isolated had similar stability profiles.

pH stability. Four pH levels (1, 3, 5, and 7) were selected. The pH 1 buffer, HCl/KCl buffer solution was prepared by mixing 67.0 mL of 0.2 M KCl with 3.5 mL 0.1 M sodium citrate and adjusting to 100 mL with deionized water. The pH 3.0 buffer was prepared by mixing 46.5 mL of 0.1 M citric acid with 3.5 mL 0.1 M sodium citrate and adjusting to 100 mL with deionized water. For pH 5.0 buffer, 20.5 mL of 0.1 M citric acid was mixed with 29.5 mL of 0.1 M sodium citrate and the solution was adjusted to 100 mL with deionized water. pH 7 buffer was made by mixing 29.1 mL of 0.1 M potassium phosphate and 29.5 mL of 0.1 M sodium hydroxide and diluting to 100 mL with deionized water. Buffer solutions had either potassium sorbate (pH 1 to 5) or sodium propionate (pH 7) as antimicrobial preservatives. Each sample was predissolved in 50% methanol solution, and aliquots (0.1 mL) diluted with 9.9 mL buffer solution at a given pH to obtain working sample.

Bisulfite stability. The method previously reported (Ojwang and Awika 2010) was used with minor modifications. Each sample was accurately weighed and dissolved in 50% aqueous methanol adjusted to pH 1.0 with HCl to produce a concentrated stock solution. From the concentrated solutions, 0.1 mL aliquot was adjusted to 10 mL with appropriate buffer solutions to produce the working control samples. The pH of each working solutions was confirmed with a pH meter. Working sample molar concentrations were apigeninidin, 6.2×10^{-5}; and for the newly identified dimeric pgiment, 5.1×10^{-5}.The absorbance spectra of these samples were recorded after 1 h (day 0), 24 h, 7, 14, 21, and 28 d, by scanning from 200 to 700 nm using a Shimadzu UV2450 UV-Vis Spectrophotometer (Shimadzu Corp., Tokyo, Japan).

For sulfite stability test, concentrated sodium metabisulfite solution was prepared using deionized water and aliquots added to the buffer solutions (5% v/v) to achieve a final SO_2 concentration of 2.52×10^{-3} M. The pH of buffer + metabisulfite solutions were checked and adjusted as appropriate with HCl or NaOH. These solutions (9.9 mL) were then mixed with equilibrated 3-deoxyanthocyanidin solutions (0.1 mL) to achieve pigment concentrations similar to control samples mentioned above. The samples were then vortexed for 30 s and UV-Vis spectra recorded after 60 min (as day 0). Additional spectra were recorded after 24 h, 7, 14, and 21 d. The initial SO_2:pigment molar ratios were >40:1 for each sample. Samples were kept at room temperature

in transparent, capped test tubes throughout analyses. All analyses were repeated 3 times. Analysis of variance was used to detect differences in the stability tests; Tukey's HSD was used for post ANOVA mean separation.

Results and Discussion

LC-MS analysis and identity of dimeric 3-deoxyanthocyanidins

Unusual pigment peaks that were less polar than typically observed in sorghum grain were present in HPLC profiles (at 480 nm) of sorghum leaf sheath extracted in aqueous methanol (data not shown). To aid in identity of these compounds, the aqueous methanol extract was partitioned with ethyl acetate to concentrate the less polar pigments. The HPLC profile of the ethyl acetate fraction revealed 4 major peaks absorbing in the UV-Vis region, labeled as a, b, c, and d (Figure 1). All these peaks had absorbance maxima at 472 to 481 nm (Table 1) that suggested they were apigeninidin-based compounds. Peaks "a" and "b" were identified as apigeninidin and 7-O-methylapigeninidin based on matching UV-Vis spectra and elution profiles with authentic standards. Additionally, the *m/z* of these compounds confirmed their identity (Table 1).

The 2 other major peaks (c and d) eluted later than the apigeninidin monomers on the reversed phase column, indicating they were less polar (Figure 1). The UV-Vis spectra of these peaks showed bathochromic shifts (3 and 9 nm) compared to the apigeninidin-based monomers (Table 1). This characteristic has been previously reported for anthocyanin dimers (Fossen and others 2004; González-Paramás and others 2006) in relation to their monomers. The MS data of compounds "c" and "d" in positive mode showed *m/z* 509 and 523, respectively (Table 1), which suggests that these compounds were dimeric flavonoid molecules differing from each other by one methyl group. Coupled with the UV-Vis data and LC elution profiles, the compounds were inferred to be 3-deoxyanthocyanidin dimers.

Fragmentation of peak "c" (*m/z* 509) led to 2 major product masses at *m/z* 384 (–125 amu) and *m/z* 255 (Figure 2A). The 1st fragment corresponds to heterocyclic ring fission (HRF) and a loss of phloroglucinol moiety, which indicates the A-ring of the dimer is 5,7-hydroxylated in the top unit (Figure 3; Vidal and others 2004; González-Paramás and others. 2006). The *m/z* 255 was the product of quinone-methide (QM) fission, which is due to the breakage of the interflavan linkage between the 2 flavonoid units (Li and Deinzer 2007; Figure 3). The fact that only one fragment mass was apparent after QM fission indicates that the terminal (bottom) and top unit fragments had the same *m/z* in the positive mode. Since the terminal unit of identified oligomeric anthocyanins contains the flavylium cation (Vidal and others. 2004; González-Paramás and others. 2006), we identified the bottom unit of compound "c" as apigeninidin moiety. Thus the top unit is likely a 3-deoxyflavonoid compound with one

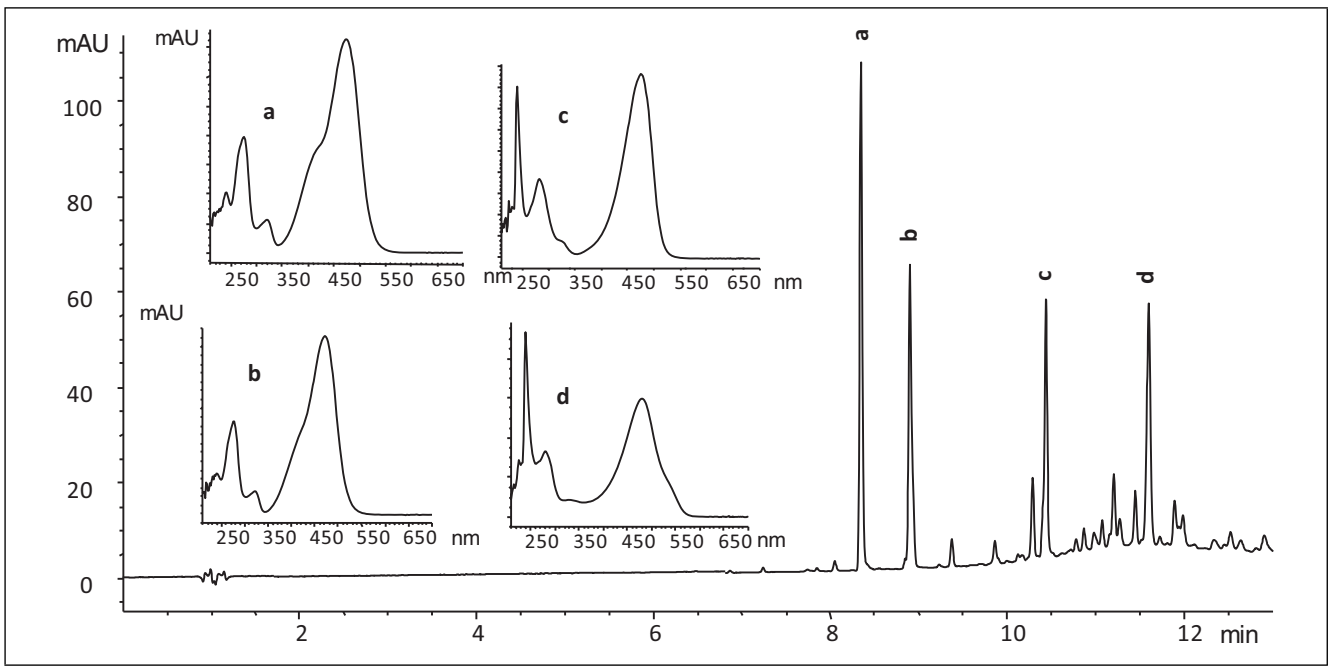

Figure 1–HPLC-DAD chromatogram and UV-Vis spectra of ethyl acetate fraction of *Sorghum bicolor* leaf sheath extract monitored at 480 nm. Peaks "a" and "b" matched spectra and elution profiles of apigeninidin and 7-O-methylapigeninidin standards, respectively. Peaks "c" and "d" were identified as new dimeric 3-deoxyanthocyanidin compounds (see Table 1).

Table 1–HPLC, UV-Vis, and mass spectra data of the major pigments identified in sorghum leaf sheath ethyl acetate extract.

HPLC peak ID	Retention time (min)	Vis λ_{max} (nm)	[M + H]+ (*m/z*)	Amount in leaf sheath powder[a] (mg/g)	Compound ID
a	8.3	472	255	48.6 ± 3.06	Apigeninidin
b	8.9	472	269	1.71 ± 0.02	7-O-methylapigeninidin
c	10.4	475	509	7.70 ± 0.65	Apigeninidin-flavene dimer
d	11.6	481	523	2.80 ± 0.28	Apigeninidin-7-O-methylflavene dimer

[a]Determined from HPLC peak areas of original aqueous methanol extract; values ± SEM (db) based on 3 separate extractions.

hydroxyl group in the B-ring, and one double bond in the C-ring. The most likely position of the hydroxyl unit on the B-ring is C-4′, based on reported 3-deoxyflavonoids in sorghum (Dykes and others 2009). The most common B-type flavonoid dimers are linked via 4→8 and, to a lesser extent, 4→6 covalent bonds. However, in mass spectroscopy these 2 types of linkages can be readily distinguished by the relative collision energy required to fragment them; the 4→6 linkage generally requires much higher collision energy to fragment (usually ≥60%) than was used in this study (33%; Li and Deinzer 2007). Thus we can conclude that this compound is a 4→8 covalently linked apigeninidin-flavene dimer, possibly a natural apigeninidin-apigenin condensation product (Figure 3). Apigeninidin and apigenin are known to coexist in specific sorghum varieties as monomers (Dykes and others. 2009), and in fact we detected significant quantities of apigenin in the sample used in this assay (data not shown). However, this is the 1st time the apigeninidin-apigenin condensation product is reported in sorghum or any other natural source.

Peak "d" (*m/z* 523), on the other hand, produced 3 distinguishable daughter ions (Figure 2B). The 1st fragment at *m/z* 384 (–139 amu) corresponds to HRF as reported for compound "c" earlier, but with a loss of phloroglucinol monomethyl ether unit. This indicates that the top unit of this dimer has one hydroxyl and one O-methyl group in the A-ring (Figure 3). HRF of the terminal unit is possible, but this would lead to a loss of the B-ring of this unit (–133 amu) instead (Li and Deinzer 2007), which was not the case. This rules out the presence of the O-methyl group on the terminal (apigeninidin) unit. The QM fission was responsible for the other MS/MS ions at *m/z* 269 (methoxylated quinone methide unit) and 255 (apigeninidin unit); these ions confirm that

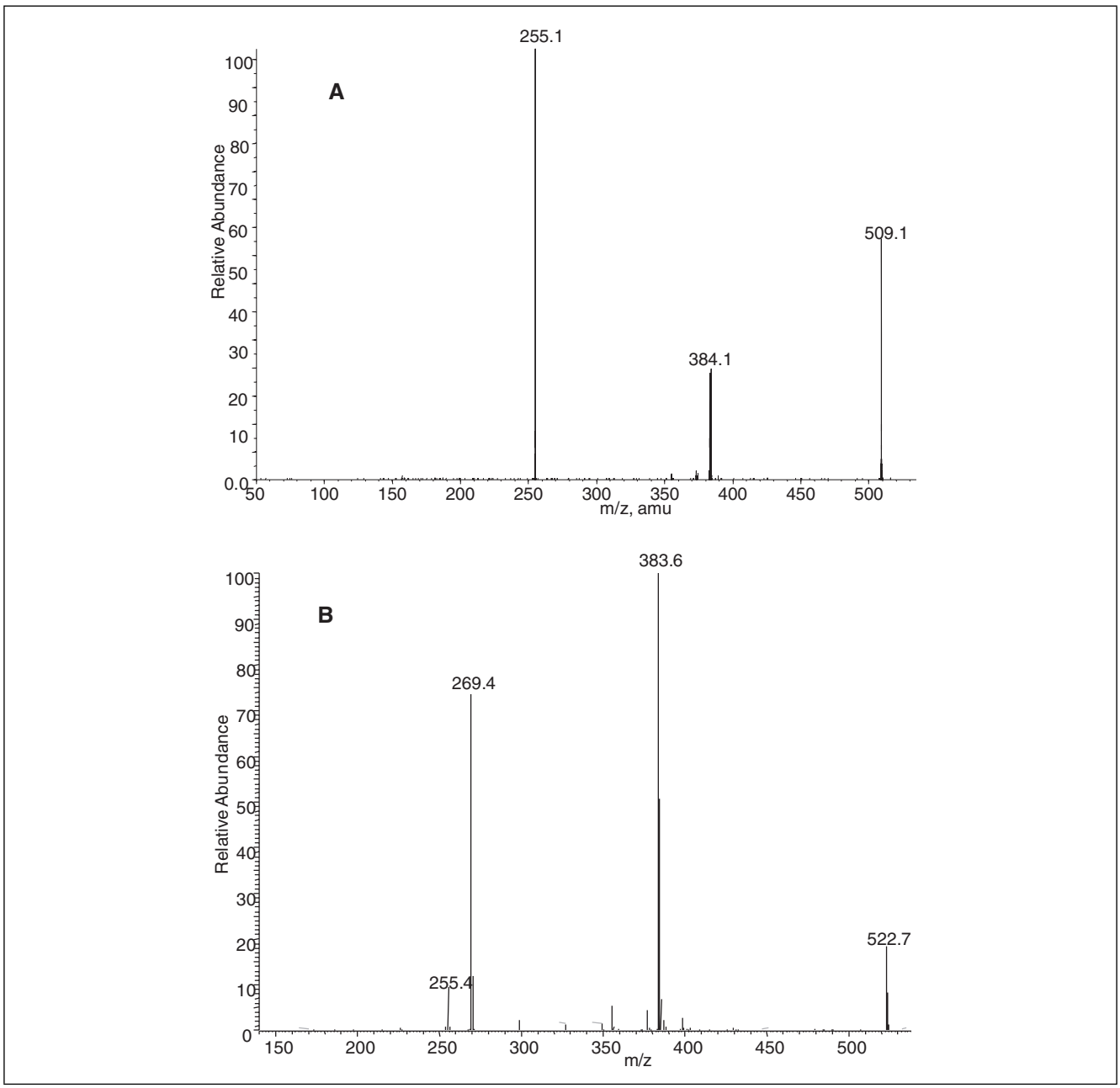

Figure 2–MS/MS spectra of compounds "c," *m/z* 509 (A) and "d," *m/z* 523 (B).

compound "d" was similar to compound "c" with the exception of the O-methyl substituent in the top unit of "d." Based on the low collision energy required for fragmentation (Li and Deinzer 2007), we propose this compound to be a 4→8 covalently linked apigeninidin-7-O-methylflavene dimer. This is the 1st report of such a compound in sorghum or in nature.

Content of the isolated pigments in sorghum leaf sheath

The predominant pigment in the sorghum leaf sheath powder was apigeninidin (48.5 mg/g; Table 1), which agrees with a recent report for similar sorghum leaf sheaths (Kayodé and others. 2011). These authors reported apigeninidin as the major pigment in sorghum leaf sheaths from local dye sorghum landraces in Benin, West Africa, with values ranging from 14.8 to 45.7 mg/g. The newly identified dimeric pigments constituted 7.7 mg/g and 2.8 mg/g, for apigeninidin-flavene and apigeninidin-7-O-methylflavene, respectively, in the sorghum powder (Table 1). These values are relatively high compared to other known natural sources of 3-deoxyanthocyanidins. For example, we previously reported that the levels of the major pigments, apigeninidin and luteolinidin, in black sorghum bran ranged between 0.4 and 1.6 and 0.5 and 1.8 mg/g, respectively (Awika and others. 2005). Thus the pigmented sorghum leaf sheath is a promising source of the stable pigments.

Color properties of dimeric 3-deoxyanthocyanidins

Even though dimeric and other polymeric anthocyanin pigments have recently been reported in other plant materials, including grape skin (Vidal and others. 2004) and purple corn (González-Manzano and others 2008), little is known about their chromatic behavior relative to the monomeric anthocyanins. This is partly due to the difficulty of isolating these pigments using standard techniques. In our case we effectively isolated high purity dimeric 3-deoxyanthocyanidin pigments using semipreparative HPLC, which enabled us to establish some interesting properties relative to their monomeric analogs. A comparison between the apigeninidin-flavene dimer (compound "d") and apigeninidin is discussed later.

Effect of pH

The 3-deoxyanthocyanins are known to resist fading or change in chromatic properties due to change in pH much better than anthocyanins, particularly at pH values ≤3.0 (Awika and others. 2004). However, at pH ≥ 5.0, formation of quinoidal bases and chalcone species become more apparent, thus visibly altering

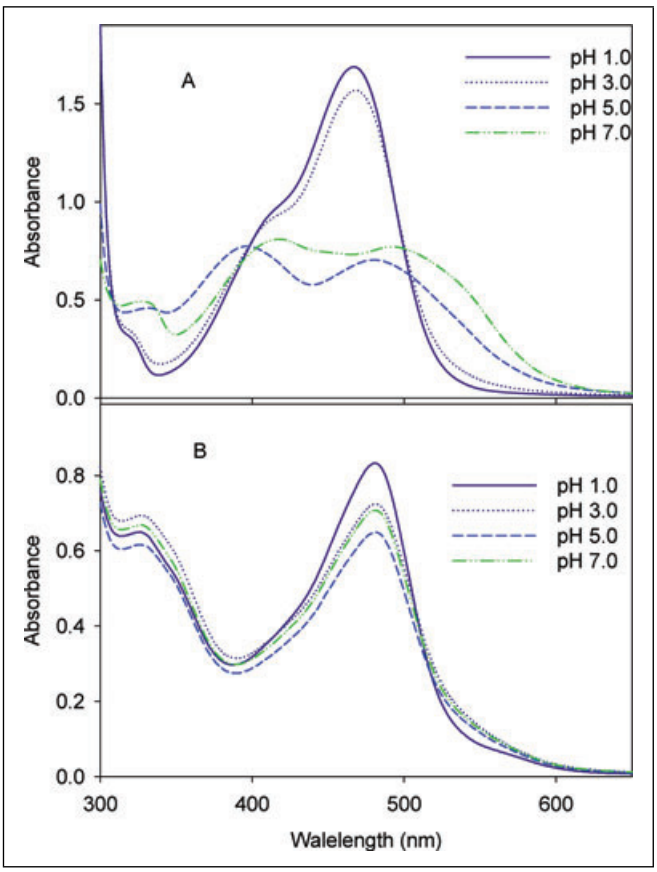

Figure 4–UV-Vis absorbance profile of apigeninidin (A) compared to the apigeninidin-flavene dimer (compound "c") (B) in the pH range 1.0 to 7.0.

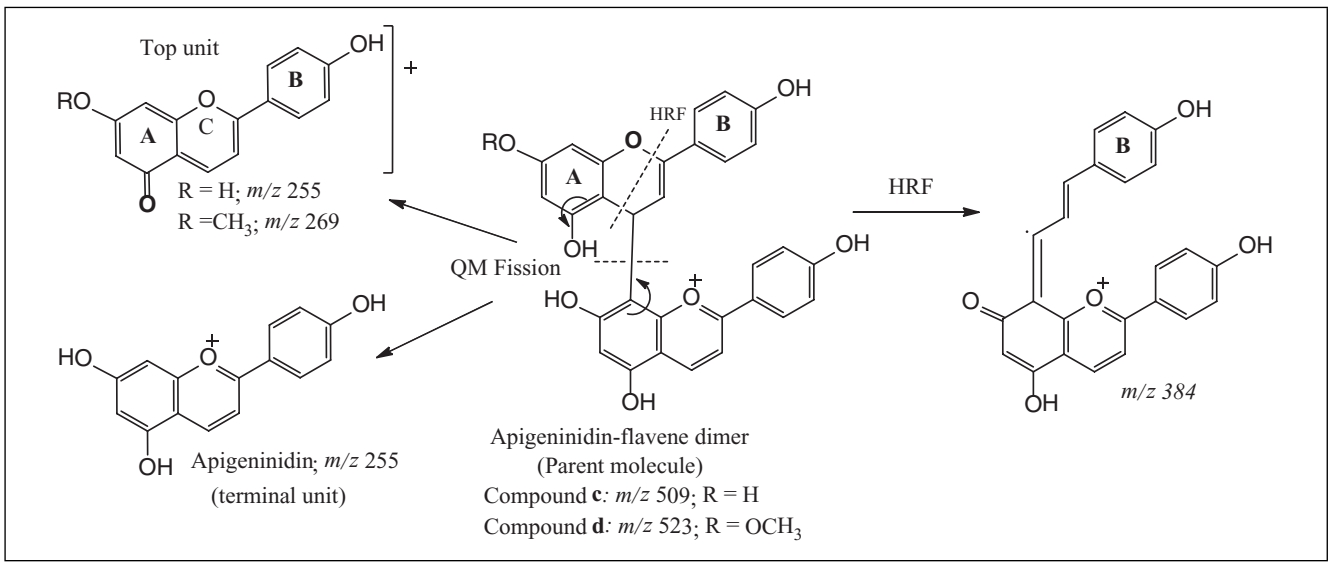

Figure 3–Major fragmentation patterns of 3-deoxyanthocyanidin dimers with B-type linkage identified in *Sorghum bicolor* sheath extract; positive ionization, ESI-MS/MS. QM = quinone methide; HRF = heterocyclic ring fission.

Table 2–Stability of apigeninidin-flavene dimer relative to apigeninidin in bisulfite solution at pH 1.0 to 7.0 during a 28-d period.

	pH 1.0		pH 3.0		pH 5.0		pH 7.0	
	DAPG[a]	APG[b]	DAPG	APG	DAPG	APG	DAPG	APG
Day 0	91 ± 2.7	19 ± 1.6	83 ± 1.0	9.1 ± 0.3	85 ± 0.9	18 ± 0.5	86 ± 0.2	20 ± 0.2
Day 1	89 ± 1.3	18 ± 1.2	82 ± 1.1	8.7 ± 0.1	85 ± 1.0	18 ± 0.2	85 ± 0.1	19 ± 0.1
Day 7	91 ± 3.5	28 ± 2.5	83 ± 1.2	19 ± 0.7	85 ± 0.6	20 ± 0.2	89 ± 0.9	27 ± 1.2
Day 14	91 ± 3.9	58 ± 7.5	87 ± 0.9	32 ± 6.2	86 ± 1.4	23 ± 0.8	91± 0.2	42 ± 4.1
Day 21	96 ± 1.0	77 ± 1.2	88 ± 0.6	43 ± 8.3	86 ± 0.8	45 ± 4.8	94 ± 1.8	42 ± 4.6
Day 28	97 ± 0.9	78 ± 1.6	97 ± 5.3	41 ± 9.3	94 ± 14	76 ± 1.3	91 ± 9.6	48 ± 7.8

Values represent means ± SD (based on 3 separate experiments) of percent relative absorbance at λ_{max} compared to control (without bisulfite); molar ration of pigment:SO_2 was 1:>40. Samples were stored at room temperature in capped transparent vials under atmospheric conditions. Day 0 reading was taken 1 h after bisulfate addition.
[a]DAPG = dimeric apigeninidin (compound "d").
[b]APG = apigeninidin. All paired comparisons between apigeninidn and dimeric apigeninidin within each treatment (day and pH) were significant at $P < 0.05$ (Tukey's HSD).

chromatic attributes of these pigments, as can be seen for apigeninidin (Figure 4A). Surprisingly, the apigeninidin-flavene dimer did not show much change in visible absorbance profile in the pH range of 1.0 to 7.0 (Figure 4B). The lowest stability was observed at pH 5.0, where the absorbance at λ_{max} was 22% less (significant at $P < 0.05$) than absorbance at pH 1.0. The pH 3.0 and pH 7.0 samples had virtually identical spectra with a smaller (12%) drop in absorbance at λ_{max} compared to pH 1.0 sample. Other than the modest change in color intensity, the spectral properties of the pigment did not significantly change at any pH, with λ_{max} remaining the same at 481 nm. The fact that no formation of chalcones or quinoidal base peaks was apparent at the neutral and near neutral pH suggests that the flavylium cation of the apigeninidin-flavene dimer is strongly protected from hydrophilic attack even at the neutral pH. This is possibly due to the increased molecular complexity of the dimer relative to the monomer.

Acylation and other forms of substitution that increase molecular complexity are well known to impart increased stability to anthocyanins. However, a synthetic dimeric anthocyanin pigment (catechin-malvidin-3-glucoside) was reported to be no more stable than the monomeric malvidin-3-glucoside and completely faded at pH 4.0 (Salas and others 2004). This suggests that dimerization may not significantly contribute to protection of anthocyanins flavylium cation from deprotonation reactions and color degradation. In our case, we clearly demonstrate superior pigment stability of apigeninidin-flavene dimer against change in pH relative to its monomer.

The color properties (absorbance and spectral profile) of the apigeninidin-flavene molecule did not change over a 4 wk period (28 d) of monitoring at room temperature further confirming its stability. Anthocyanin-based compounds that do not change color with change in pH have not been previously reported. Thus the behavior observed for the apigeninidin-flavene dimer is most unusual and deserves further investigation. It is also remarkable because it suggests these dimeric 3-deoxyanthocyanins could be useful as very stable colors with predictable hue properties over a wide pH range.

Effect of sodium metabisulfite

With the observed resistance to stability to changing pH, we were further interested in understanding how the dimeric apigeninidin-flavene dimer would withstand nucleophilic attack by bisulfite. This was important given we recently observed rapid (within 24 h) and near complete bleaching of monomeric apigeninidin derivatives in the presence of bisulfite at pH values between 1.8 and 5.0 (Ojwang and Awika 2010). The apigeninidin-flavene dimer showed significantly ($P < 0.05$) stronger resistance to bleaching by bisulfite compared to apigeninidin at all pH levels studied (Table 2). The maximum drop in absorbance at λ_{max} was observed after 24 h, with an average loss of absorbance of 14.5% for the pH range of 1.0 to 7.0 in the presence of excess bisulfite (1: 40 pigment:SO_2 molar ratio) relative to control (Table 2). By comparison, the monomeric apigeninidin showed the expected susceptibility to nuceophilic attack, losing an average of 72% to 92% absorbance at λ_{max} within 24 h in presence of sulfite, similar to recent findings (Ojwang and Awika 2010). Previous report using crude polymeric anthocyanin extracts from grape skin indicated a loss of 55% of absorbance in presence of metabisulfite (Vidal and others. 2004); an even lower stability is likely for purified polymeric anthocyanins. The apigeninidin-flavene compound remained stable over a period of 28 d in the presence of bisulfite at room temperature, gradually regaining most of the absorbance that was initially lost (Table 2). Thus the protective effect of 3-deoxyanthocyanin dimerization against color degradation is superior to that observed for anthocyanin dimers.

Conclusion

The resistance of the dimeric 3-deoxyanthocyanidin molecule to beaching effect of SO_2 demonstrates that dimerization significantly ($P < 0.05$) protects the 3-deoxyanthocyanins from nucleophiles. The observed color stability in a wide pH range further indicates these compounds are relatively resistant to deprotonation, that is, the intensely colored flavylium cation is the favored species even at neutral pH. The dimeric 3-deoxyanthocyanins could be useful as stable colors with predictable hue properties over a wide pH range, and in the presence of common food additives like bisulfate that negatively impact anthocyanin stability. Thus these compounds present interesting new opportunities for using natural pigments in foods. Understanding how sorghum accumulates the complex pigments should be investigated as this may lead to future optimization of their accumulation in large quantities in the plant for food applications.

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