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Antioxidant Capacity, Phenolic Content, and Profiling of Phenolic Compounds in the Seeds, Skin, and Pulp of *Vitis rotundifolia* (Muscadine Grapes) As Determined by HPLC-DAD-ESI-MS^n

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The objective of this study was to determine and compare the total phenolic content and antioxidant capacity in the seeds, skin, and pulp of eight cultivars of Florida-grown muscadine grapes and to identify the phenolic compounds in these respective portions. Total phenolic contents were determined colorimetrically using Folin-Ciocalteu reagent, and antioxidant capacity was determined by oxygen radical absorbance capacity (ORAC). High-performance liquid chromatography equipped with diode array (HPLC-DAD) and electrospray ionization mass spectrometric detection (ESI-MS^n) was used to identify the phenolic compounds in the seeds, skin, and pulp of muscadine grapes. The total phenolic content and antioxidant capacity, based on fresh weight, were highest in seeds followed by skin and pulp. On average, 87.1, 11.3, and 1.6% of phenolic compounds were present in seeds, skin, and pulp, respectively. A total of 88 phenolic compounds of diverse structures were tentatively identified in muscadines, which included 17 in the pulp, 28 in the skin, and 43 in the seeds. Seventeen compounds were identified for the first time in muscadine grapes. The compounds identified in seeds included hydrolyzable tannins, flavan-3-ols and condensed tannins, ellagic acid derivatives, and quercetin rhamnoside. The skin contained hydrolyzable tannins, flavonoids, including anthocyanin 3,5-diglucosides, quercetin, myricetin, and kaempferol glycosides.

KEYWORDS: Muscadine grapes; hydrolyzable tannins; ellagic acid; flavonols

INTRODUCTION

Phenolic compounds make up a class of phytochemicals that play an important role in the nutritional and sensory properties of various fruits and vegetables. They are categorized into different classes depending upon their structures (**Figure 1**), varying from simple phenolic acids (hydroxybenzoic acid and hydroxycinnamic acid) to complex polyphenols (hydrolyzable and condensed tannins) (*1*, *2*). Phenolic compounds have been linked to many positive health benefits, including protective effects against certain diseases such as cancer and cardiovascular diseases (*3*−*5*). The protective effect of phenolic compounds has been attributed in part to their antioxidant capacity (*6*, *7*).

Muscadine grapes are commonly grown in the southeastern United States and are well-adapted to warm, humid climates, which are unsuitable for the growth of other grapes (*Vitis vinifera*). They are either light-skinned (green or bronze) or dark-skinned (red to almost black) (*8*−*10*) and are 1−1.5 in. in diameter with thick, tough skin that protects them from heat, UV radiation, humidity, insects, and fungi. They grow in tight small clusters of 3−10 berries and are marketed in fresh and processed forms such as juice, wine, and jam.

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Muscadine grapes contain a large variety of antioxidant phytochemicals. They are reported to contain hydroxybenzoic acids, ellagic acid in free and conjugated form, resveratrol, and flavonoids, including anthocyanins, quercetin, myricetin, and kaempferol (*10*−*12*). Cell culture studies have suggested that polyphenols from muscadine grapes can inhibit proliferation of colon cancer cells and induce apoptosis in them (*13*, *14*). However, the phytochemical profiles of muscadine grapes have been documented in only a few studies. The phenolic compounds in muscadine grapes have been quantified after acid hydrolysis of the samples, which limits their actual structural identification (*8*). Two other studies identified the phenolic compounds in the skin of muscadine grapes (*11*, *12*); however, specific information about the identification of phenolic compounds in the seeds and pulp of muscadine grapes is lacking. High-performance liquid chromatography coupled with a diode array detector and a mass spectrometry (HPLC-DAD-MS^n) provides a powerful tool for phytochemical analysis in crude plant extracts. It provides useful structural information and allows for tentative compound identification when standard reference compounds are unavailable and when peaks have similar retention times and UV absorption spectra (*15*, *16*). The aim of our study was to evaluate the antioxidant capacity and total phenolic content and to perform an extensive identification of the phenolic compounds in the seeds,

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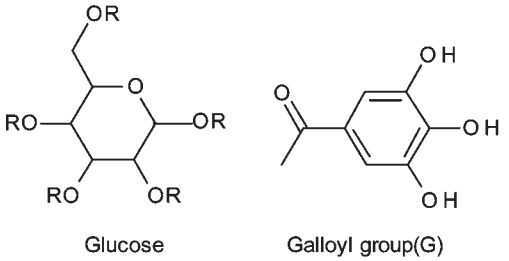
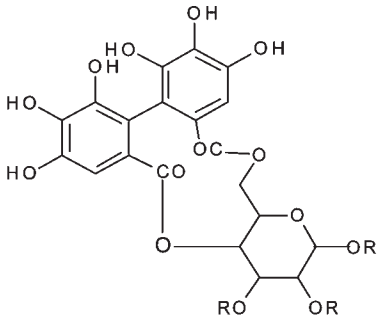
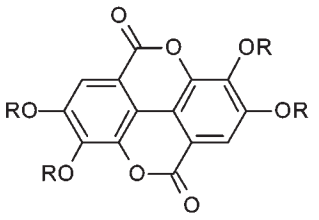


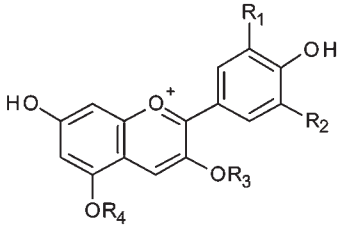
Hydrolyzable tannins	R
Monogalloyl glucose	Four R's are H atoms and one R is G group
Digalloyl glucose	Three R's are H atom and two R's are G group
Trigalloyl glucose	Two R's are H atom and three R's are G group
Tetragalloyl glucose	One R is H atom and four R's are G group
Pentagalloyl glucose	All R's are G group
Hexagalloyl glucose	Another G group attaches with pentagalloyl glucose



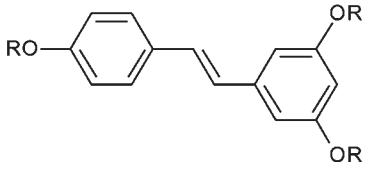
Hydrolyzable tannins	R
HHDP-glucose	H
Galloyl-HHDP-glucose	Any R could be G



Ellagic acid and conjugates	R
Ellagic acid	H
Ellagic acid hexoside	Any one R could be hexose
Ellagic acid xyloside	Any one R could be xylose
Ellagic acid rhamnoside	Any one R could be rhamnose



Anthocyanins	R_1	R_2	R_3	R_4
Delphinidin 3,5-diglucoside	OH	OH	glucose	glucose
Cyanidin 3,5-diglucoside	OH	H	glucose	glucose
Petunidin 3,5-diglucoside	OCH_3	OH	glucose	glucose
Pelargonidin 3,5-diglucoside	H	H	glucose	glucose
Peonidin 3,5-diglucoside	OCH_3	H	glucose	glucose
Malvidin 3,5-diglucoside	OCH_3	OCH_3	glucose	glucose



Stilbenes	R
Resveratrol	H
Resveratrol glucoside	Any R could be glucose

Figure 1. Proposed structures of phenolic compounds in muscadine grapes.

skin, and pulp of eight cultivars of Florida-grown muscadine grapes using a simple and rapid high-performance liquid chromatography and mass spectrometry (HPLC-MS^n) technique. Seventeen different phenolic compounds were identified for the first time in muscadine grapes. The comprehensive knowledge of phenolic compounds in the seeds, skin, and pulp of muscadine grapes can contribute to a better understanding of their influence on the quality of muscadine products, especially wine and juice.

MATERIALS AND METHODS

Chemicals and Materials. Ellagic acid, (+)-catechin, (−)-epicatechin, quercetin 3-*O*-*β*-glucoside, (−)-catechin gallate, (−)-gallocatechin, and (−)-epigallocatechin were purchased from Sigma-Aldrich (St. Louis, MO). Quercetin 3-*O*-*β*-rhamnoside was purchased from Indofine (Hillsborough, NJ). (−)-Epicatechin gallate and *trans*-resveratrol 3-*O*-*β*-glucoside were purchased from Chromadex (Irvine, CA). AAPH [2,2′-azobis(2-amidinopropane)] was a product of Wako Chemicals Inc. (Bellwood, RI). Gallic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), HPLC-grade methanol, acetic acid, formic acid, Folin-Ciocalteu reagent, flourescein, 96-well black plates with clear bottom wells and a lid, and sodium carbonate were purchased from Fischer Scientific Co. (Pittsburgh, PA). Eight cultivars of muscadine grapes, four bronze (Doreen, Fry, Carlos, and Triumph) and four black (Southland, Magoon, Alachua, and Noble), were obtained from a local vineyard in central Florida. The grapes were manually separated into seeds, skin, and pulp within 2 h of harvest, and the separated portions were kept at −20 °C until further analysis.

Sample Preparation. The seeds, skin, and pulp of grapes were freeze-dried and ground to a fine powder using a Waring kitchen blender. One gram of freeze-dried pulp and 0.5 g of seed or skin were weighed in 20 mL screw-capped glass tubes. The weighed samples were extracted with 10 mL of an acetone/water/acetic acid mixture (acetone/H_2O/acetic acid, 70:29.7:0.3, v/v) solvent. The extraction tubes were vortexed for 30 s, sonicated for 5 min, kept at room temperature for 20 min, and sonicated for an additional 5 min. The tubes were centrifuged at 3000 rpm for 10 min,

and the supernatant was collected in separate glass tubes. For the identification of phenolic compounds, the same extraction procedure was followed. The collected supernatant was evaporated in a SpeedVac Concentrator (Thermo scientific ISS110, Waltham, MA) under reduced pressure at 25 °C to remove the solvent. The solids obtained after evaporation were dissolved in 5 mL of 70% acidified (1% formic acid) methanol and sonicated for 5 min to resuspend the solid residue. The samples were filtered through a 0.45 μm filter prior to injection (20 μL) into the HPLC system. All the prepared samples were kept at −20 °C until they were analyzed.

HPLC-DAD-ESI-MS^n Analysis. Chromatographic analyses were performed on an Agilent 1200 series HPLC system (Agilent, Palo Alto, CA) equipped with an autosampler/injector and diode array detector. A Zorbax Stablebond Analytical SB-C_{18} column (4.6 mm × 250 mm, 5 μm, Agilent Technologies, Rising Sun, MD) was used for separation. Elution was performed using mobile phase A (0.5% formic acid aqueous solution) and mobile phase B (methanol). UV−vis spectra were scanned from 220 to 600 nm on a diode array detector with detection wavelengths of 280, 360, and 520 nm. The flow rate was 1 mL/min, and the following linear gradient was used: 5% B from 0 to 2 min, 5 to 20% B from 2 to 10 min, 20 to 30% B from 10 to 15 min, 30 to 35% B from 15 to 20 min, 80 to 85% B from 60 to 65 min, and 85 to 5% B from 65 to 70 min followed by re-equilibration of the column for 5 min for the next run. Electrospray mass spectrometry was performed with a HCT ion trap mass spectrometer (Bruker Daltonics, Billerica, MA). Column effluent was monitored in the positive and negative ion mode of the instrument in an alternative manner during the same run. Other experimental conditions for the mass spectrometer were as follows: nebulizer, 45 psi; dry gas, 11.0 L/min; dry temperature, 350 °C; ion trap, scan from m/z 100 to 2200; smart parameter setting (SPS), compound stability, 50%; trap drive level, 60%. The mass spectrometer was operated in Auto MS^3 mode. MS^2 was used to capture and fragment the most abundant ion in full scan mass spectra, and MS^3 was used to fragment the most abundant ion in MS^2.

Folin-Ciocalteu Assay. The acetone/H_2O/acetic acid extracts were diluted to the appropriate concentration for analysis. The total phenolic content was determined by using a modified method of Singleton and Rossi (*17*). The extracts were mixed with diluted Folin-Ciocalteu reagent and 15% sodium carbonate. Absorption at 765 nm was measured in a microplate reader (SPECTRAmax 190) after incubation for 30 min at room temperature. The results were expressed as milligrams of gallic acid equivalents per gram of fresh weight (mg of GAE/g) using a standard curve generated with 100−600 mg of gallic acid per liter.

Oxygen Radical Absorbance Capacity (ORAC). The ORAC assay for extracted samples was conducted on a Spectra XMS Gemini plate reader (Molecular Devices, Sunnyvale, CA). Briefly, 50 μL of standard and samples were added to the designated wells of a 96-well black plate. This was followed by addition of 100 μL of flourescein (20 nM). The mixture was incubated at 37 °C for 7 min before the addition of 50 μL of AAPH. Fluorescence was monitored using 485 nm excitation and 530 nm emission at 1 min intervals for 40 min. Trolox was used to generate a standard curve. The antioxidant capacities of extracts were expressed as micromoles of Trolox equivalents (TE) per gram of fresh weight (μmol of TE/g).

Statistical Analysis. One-way analyses of variance (ANOVA) with Tukey-HSD pairwise comparison of the means were performed using JMP (version 7.0, SAS Institute Inc., Cary, NC). Total phenolic and ORAC values are expressed as means ± the standard deviation of three independent observations. Data points from two samples were omitted as outliers on the basis of the Q-test (*18*), and the results from those values are expressed as duplicates. A $p \leq 0.05$ difference is considered significant.

RESULTS AND DISCUSSION

The phytochemical constituents of the muscadine grape (*Vitis rotundifolia*) differ from *V. vinifera* in many aspects. The presence of ellagic acid in muscadine grapes is unique, and it is found in the form of free ellagic acid, ellagic acid glycosides, and ellagitannins (*19*). Another unique feature is the fact that the anthocyanins are present as 3,5-diglucosides (as opposed to 3-glucosides in *V. vinifera*) of delphinidin, cyanidin, petunidin, peonidin, pelargonidin, and malvidin in nonacylated forms. No condensed tannins were identified in muscadine skin as opposed to *Vinifera* skin (*20*). Red muscadine wines are more susceptible to browning and loss of color after aging. This may be due to slow association of anthocyanin diglucosides and tannins present in muscadine wines (*21*).

Total Phenolic Content and Antioxidant Capacity. Table 1 lists the total phenolic contents and antioxidant capacities in eight cultivars of Florida-grown muscadine grapes. On the basis of fresh weight, the total phenolic content was highest in seeds (27.0−81.2 mg of GAE/g), followed by skin (4.3−10.2 mg of GAE/g) and pulp (0.3−1.2 mg of GAE/g). Among the seeds, the total phenolic content was highest in Alachua and lowest in Magoon muscadine grapes. The skin of the Carlos variety had the highest total phenolic content compared to the skin of other varieties. Accordingly, the antioxidant capacity, based on fresh weight, was also highest in seeds (276.6−1538.4 μmol of TE/g), followed by skin (26.0−77.5 μmol of TE/g) and pulp (2.3−4.6 μmol of TE/g). The ORAC value was found to be highest in Fry seed, Noble skin, and pulp. On average, the phenolic content of seeds, skin, and pulp was 87.1, 11.3, and 1.6% of that in whole grapes, respectively. Similarly, the average antioxidant capacity among all the grape cultivars was 93.9% in seeds, 5.6% in skin, and 0.5% in pulp. The correlation coefficient (r) between the total phenolic content and antioxidant capacity in the seeds, skin, and pulp of eight cultivars of muscadines was 0.87.

Phenolic Identification via HPLC-DAD-ESI-MS^n. Identification of phenolic compounds was accomplished in both the bronze and black cultivars of muscadine grapes. Because of the similarity of phenolic compounds in each grape cultivar, the identification data from only one variety (cv. Noble) are discussed; however, the few differences detected among grape cultivars are also reported. The HPLC-DAD chromatograms of muscadine grape pulp, skin, and seed were recorded at 280, 360, and 520 nm (Figures S1−S3 of the Supporting Information). Most of the phenolic compounds can be detected at 280 nm. Ellagic acid derivatives and flavonols have maximum absorption at 360 nm. Anthocyanins were detected at 520 nm in the skin of black varieties. Anthocyanins were also detected in the pulp; however, it could be due to migration of these pigments from the skin to the pulp during the separation of fruit into its parts. The mass spectrometer was operated in both positive and negative ionization modes in the same HPLC run. Anthocyanins have inherent positive charge, so they have maximum sensitivity in the positive modes of the mass spectrometer; however, for most other flavonoids, the highest sensitivity was obtained in the negative ionization mode. Most flavonoids are present in nature as glycosides and other conjugates (*22*, *23*). Identification of a sugar moiety attached to phenolic compounds in muscadine grapes was based on fragmentation data from MS and previous literature reports (*12*).

Phenolic compounds in the pulp, skin, and seeds were identified on the basis of mass spectral data, chromatography of pure standards, and UV−vis spectra on the diode array detector. Seventeen phenolic compounds are reported for the first time in Noble pulp that include caffeic acid hexoside, hydrolyzable tannins, mostly gallotannins, epicatechin, epicatechin gallate, ellagic acid and its conjugates, flavonol glycosides, and isomeric forms of resveratrol glucoside (**Table 2**). In Noble skin, 28 phenolic compounds are reported, and among those, eight compounds were identified for the first time (**Table 3**). These compounds were caffeic acid hexoside, hexahydroxydiphenoic glucose (HHDP-glucose), monogalloyl glucose, ellagic acid hexoside, kaempferol rutinoside, and hexosides of myricetin, quercetin, and kaempferol. No condensed tannins were identified in the skin. Forty-three different phenolic compounds identified in Noble seeds are listed in **Table 4**. No previous study of muscadine grapes has identified these compounds. Among the various

Table 1. Total Phenolic Content and Antioxidant Capacity of the Seeds, Skin, and Pulp of Different Cultivars of Muscadine Grapes[a]

	total phenolic content (mg of GAE/g)			antioxidant capacity (ORAC, μmol of TE/g)		
cultivar	seeds	skin	pulp	seeds	skin	pulp
			Bronze			
Doreen	45.1 ± 5.3 b	4.5 ± 0.4 cd	1.0 ± 0.0 b	797.9 ± 29.8 c	26.0 ± 5.3 d	4.0 ± 0.3 ab
Fry	68.8 ± 5.9 a	4.7 ± 0.3 cd	0.9 ± 0.0 bc	1538.4 ± 41.8 a	*37.9 ± 0.3 bcd	4.0 ± 0.3 ab
Carlos	37.4 ± 2.0 bc	10.2 ± 0.6 a	0.8 ± 0.0 c	499.6 ± 8.2 de	43.1 ± 0.6 b	2.4 ± 0.3 c
Triumph	40.0 ± 7.7 bc	4.3 ± 0.5 d	0.3 ± 0.0 d	530.8 ± 39.8 d	27.1 ± 3.2 cd	2.3 ± 0.4 c
			Black			
Southland	44.4 ± 2.7 b	6.2 ± 0.4 bc	1.2 ± 0.1 a	313.9 ± 2.5 f	*43.9 ± 0.2 b	3.6 ± 0.2 b
Magoon	27.0 ± 2.2 c	5.9 ± 0.6 bcd	0.9 ± 0.0 bc	432.2 ± 16.5 e	37.9 ± 1.0 bc	3.4 ± 0.3 b
Alachua	81.2 ± 5.8 a	6.1 ± 0.7 bc	0.9 ± 0.0 bc	1105.4 ± 8.8 b	42.1 ± 1.8 b	3.3 ± 0.3 b
Noble	36.6 ± 5.6 bc	7.5 ± 0.8 b	0.9 ± 0.0 bc	276.6 ± 18.3 f	77.5 ± 8.1 a	4.6 ± 0.1 a

[a] Results are means ± the standard deviation of three determinations on a fresh weight basis. Values preceded by an asterisk are duplicates due to rejection of data points based on the Q-test. Different letters in each column indicate the significant differences in the mean at the $p \leq 0.05$ level.

Table 2. Retention Times and Mass Spectrometric Data of Phenolic Compounds in Muscadine Grape Pulp (cv. Noble) Determined by HPLC-ESI-MS^n

compound	retention time (t_R) (min)	molecular weight	MS^1 (*m/z*)	MS^2 (*m/z*)[a]	MS^3 (*m/z*)[a]	identified compound
			Hydroxycinnamic Acid Derivatives			
1	3.1	342	377 [M + Cl^-], [341 + 36]	**341**, 215	179, **161**, 113, 101	caffeic acid hexoside*
			Hydrolyzable Tannins			
4	5.7	332	331 [M − H]$^-$	313, 271, **169**, 125		monogalloyl glucose*
6	7.2	332	331 [M − H]$^-$	271, 211, **169**, 125		monogalloyl glucose
13	9.4	494	493 [M − H]$^-$	456, 377, **331**, 169, 157		monogalloyl diglucose*
			Flavan-3-ols			
46	20.4	290	289 [M − H]$^-$	**245**, 227, 205, 179, 137, 109	**203**, 188, 161, 123	epicatechin#
53	24.2	442	441 [M − H]$^-$	**289**, 169, 125	**245**, 230, 203, 179, 107	epicatechin gallate*#
			Ellagic Acid and Conjugates			
55	25.6	464	463 [M − H]$^-$	**301**, 284, 257, 229, 217		ellagic acid hexoside*
63	31.7	434	433 [M − H]$^-$	301	**284**, 257	ellagic acid xyloside
65	32.9	448	447 [M − H]$^-$	**301**, 299, 257	300, **185**	ellagic acid rhamnoside
67	34.0	302	301 [M − H]$^-$	262, **257**		ellagic acid#
			Flavonols			
54	25.4	594	593 [M − H]$^-$	535, 447, **285**		kaempferol rutinoside*
62	30.5	464	463 [M − H]$^-$	405, **317**, 316, 271, 179	**287**, 271, 215, 179,126	myricetin rhamnoside
68	35.6	448	447 [M − H]$^-$	437, 376, 344, 329, 321, **301**, 271, 255, 228, 191, 179, 167, 151	271, **179**	quercetin 3-*O*-β-rhamnoside#
69	36.0	448	447 [M − H]$^-$	327, **285**, 257, 179, 134		kaempferol hexoside*
70	39.6	432	431 [M − H]$^-$	**285**, 255, 214, 179, 163		kaempferol rhamnoside
			Stilbenes			
50	22.9	390	425 [M + Cl^-], [389 + 36]	**389**, 227		resveratrol glucoside
56	25.7	390	425 [M + Cl^-], [389 + 36]	**389**, 227		*trans*-resveratrol 3-*O*-β-glucoside#

[a] Ions in boldface indicate the most intense product ion (100% relative intensity). Compounds followed by an asterisk were identified for the first time in muscadine grapes. Compounds followed by a superscript number sign were identified using pure standards. All other compounds were tentatively identified on the basis of mass data.

phenolic compounds in seeds, hydrolyzable tannins were most prominent. Condensed tannins and flavan-3-ols, ellagic acid conjugates, quercetin rhamnoside, and caffeic acid hexoside were also identified in Noble seeds.

Hydroxycinnamic Acid Derivatives. Compound **1** (**Tables 2**−**4** and **Figure 2**) had *m/z* 377 [M + Cl^-] ion, which indicates a chloride adduct that fragmented to yield *m/z* 341 [M - H]$^-$ as the most intense ion in MS^2. Compound with *m/z* 341 further

Table 3. Retention Times and Mass Spectrometric Data of Phenolic Compounds in Muscadine Grape Skin (cv. Noble) Determined by HPLC-ESI-MS^n

compound	retention time (t_R) (min)	molecular weight	MS^1 (m/z)	MS^2 (m/z)[a]	MS^3 (m/z)[a]	identified compound
			Hydroxycinnamic Acid Derivatives			
1	3.1	342	377 [M + Cl$^-$], [341 + 36]	**341**, 215, 179	**179**, 161, 143, 131, 125, 101	caffeic acid hexoside*
			Hydrolyzable Tannins			
3	4.2	482	481 [M − H]$^-$	421, **301**	258, 201, **185**, 175	HHDP-glucose*
6	7.3	332	331 [M − H]$^-$	271, 211, **169**, 125	125	monogalloylglucose*
11	8.8	332	331 [M − H]$^-$	**169**, 125	125	monogalloylglucose
15	9.6	634	633 [M − H]$^-$	613, 481, **301**, 275, 250, 230, 178	300, **257**	HHDP-galloylglucose
22	12.9	626	625 [M − H]$^-$	623, 481, **463**, 320, 301, 239, 193	355, 319, **301**, 275, 257, 239, 215, 193, 175,164,147	HHDP-diglucoside
47	21.0	814	813 [M − H]$^-$	781, **763**, 753, 745, 735, 725, 511, 301, 257		ellagitannin
51	23.5	832	831 [M − H]$^-$	**813**, 795, 787, 769, 752, 741, 723, 707, 697, 680, 664, 611, 578, 451, 365, 301, 291, 254		ellagitannin
52	23.8	818	817 [M − H]$^-$	**773**, 755, 729, 712, 701, 685, 673, 667, 655, 655, 647, 621, 617, 541, 503, 371, 237	**729**, 712, 701, 685, 655, 617, 577, 531, 465, 407, 301, 237	ellagitannin
			Flavan-3-ols			
18	11.8	306	305 [M − H]$^-$	285, 263, 247, **219**, 198, 179, 165, 151, 137, 125		gallocatechin#
			Anthocyanins			
27	14.7	627	627 [M]$^+$	465, **303**	285, **257**, 229, 149	delphinidin 3,5-diglucoside
29	16.1	611	611 [M]$^+$	449, **287**	269, 241, 213, **189**, 167, 149, 137, 109	cyanidin 3,5-diglucoside
33	17.1	641	641 [M]$^+$	479, **317**	**302**, 274, 218	petunidin 3,5-diglucoside
38	18.2	595	595 [M]$^+$	433, **271**	**225**, 215, 197, 187, 169, 141, 131, 121	pelargonidin 3,5-diglucoside
39	18.2	625	625 [M]$^+$	463, **301**	**286**	peonidin 3,5-diglucoside
44	20.0	655	655 [M]$^+$	493, **331**	**315**, 299, 287, 270, 243, 179	malvidin 3,5-diglucoside
			Ellagic Acid and Conjugates			
55	25.6	464	463 [M − H]$^-$	**301**	**300**, 284, 257, 157	ellagic acid hexoside*
63	31.8	434	433 [M − H]$^-$	**301**	300, 257, **245**, 229, 188, 145	ellagic acid xyloside
65	32.9	448	447 [M − H]$^-$	**301**	300, 257, 229, **216**, 160	ellagic acid rhamnoside
67	34.0	302	301 [M − H]$^-$	284, **257**, 229, 201, 173		ellagic acid#
			Flavonols			
54	25.4	594	593 [M − H]$^-$	534, 431, 333, **285**	**211**	kaempferol rutinoside*
59	28.2	480	479 [M − H]$^-$	359, **317**, 270, 179	287, **271**, 259, 227, 179, 151, 125, 109	myricetin hexoside*
62	30.4	464	463 [M − H]$^-$	**317**, 271, 179	287, 271, 242, 193, **179**, 151, 137	myricetin rhamnoside
64	32.4	464	463 [M − H]$^-$	**301**, 151	299, 271, 255, 230, 212, **179**, 151, 121	quercetin 3-*O*-β-glucoside*#
68	35.6	448	447 [M − H]$^-$	**301**	271, 255, 226, 193, 179, **151**	quercetin 3-*O*-β-rhamnoside#
69	36.0	448	447 [M − H]$^-$	**285**, 255, 227	267, **255**, 239, 227, 199, 169, 135	kaempferol hexoside*
70	39.6	432	431 [M − H]$^-$	**285**	267, **255**, 241, 229, 213, 195, 187, 174	kaempferol rhamnoside
			Stilbenes			
66	33.3	390	389 [M − H]$^-$	**227**	**185**, 157, 143	resveratrol glucoside

[a] Ions in boldface indicate the most intense product ion (100% relative intensity). Compounds followed with an asterisk were identified for the first time in muscadine grapes. Compounds followed by a superscript number sign were identified using pure standards. All other compounds were tentatively identified on the basis of mass data.

Table 4. Retention Times and Mass Spectrometric Data of Phenolic Compounds in Muscadine Grape Seeds (cv. Noble) Determined by HPLC-ESI-MS^n

compound	retention time (t_R) (min)	molecular weight	MS^1 (m/z)	MS^2 (m/z)[a]	MS^3 (m/z)[a]	identified compound
			Hydroxycinnamic Acid Derivatives			
1	3.0	342	377 [M + Cl$^-$], [341 + 36]	**341**, 215, 179, 161	**179**, 143, 131, 119, 113	caffeic acid hexoside*
			Hydroxybenzoic Acid			
8	7.8	170	169 [M − H]$^-$	**125**		gallic acid#
			Hydrolyzable Tannins			
2	3.6	482	481 [M − H]$^-$	**301**, 169	**147**	HHDP-glucose*
5	6.7	332	331 [M − H]$^-$	271, 241, 211, **169**, 125	**125**	monogalloylglucose*
7	7.4	484	483 [M − H]$^-$	**331**, 313, 169		digalloylglucose
9	8.3	332	331 [M − H]$^-$	271, **169**, 125	**125**	monogalloylglucose
10	8.6	484	483 [M − H]$^-$	**331**, 211, 169	**169**	digalloylglucose
12	8.8	634	633 [M − H]$^-$	**481**, 301	**185**	galloyl-HHDP-glucose
13	9.2	494	493 [M − H]$^-$	**331**, 169	**169**	monogalloyldiglucose*
14	9.2	634	633 [M − H]$^-$	593, 481, **301**	284, **229**	HHDP-galloylglucose
16	11.2	484	483 [M − H]$^-$	**331**, 313, 169	271, 241, 211, **169**	digalloylglucose
17	11.2	634	633 [M − H]$^-$	613, 481, **301**, 185	257, **185**	HHDP-galloylglucose
19	12.1	332	331 [M − H]$^-$	**169**, 125	**125**	monogalloylglucose
20	12.7	634	633 [M − H]$^-$	615, 481, 421, **301**, 229, 185	300, 257, 201, **187**	HHDP-galloylglucose
21	12.7	786	785 [M − H]$^-$	748, **633**, 615, 483, 331, 301, 275		HHDP-digalloylglucose*
23	13.2	634	633 [M − H]$^-$	613, 572, 483, **301**, 275, 257, 228, 201	**257**	HHDP-galloylglucose
24	13.2	786	785 [M − H]$^-$	765, **633**, 301, 275, 231, 223		HHDP-digalloylglucose
25	13.7	636	635 [M − H]$^-$	613, **483**, 331, 211	**313**, 207, 169	trigalloylglucose*
26	14.2	636	635 [M − H]$^-$	614, **483**, 301, 229	331, **169**	trigalloylglucose
28	14.8	634	633 [M − H]$^-$	613, 566, 483, **301**, 284, 257, 229, 185		HHDP-galloylglucose
30	16.2	634	633 [M − H]$^-$	465, **301**	**257**, 229	HHDP-galloylglucose
31	16.2	786	785 [M − H]$^-$	**633**, 543, 483, 301	482, **301**, 275	HHDP-digalloylglucose
32	16.5	636	635 [M − H]$^-$	597, **483**, 465, 420, 313, 193	424, 331, 313, **169**	trigalloylglucose
35	17.3	636	635 [M − H]$^-$	599, **483**, 423, 406, 332, 235, 194	405, **331**, 313, 271, 211, 169	trigalloylglucose
37	18.1	786	785 [M − H]$^-$	768, **633**, 615, 596, 465, 419, 301, 285	313, **301**, 275, 214	HHDP-digalloylglucose
40	19.4	788	787 [M − H]$^-$	**635**, 483	**617**, 483, 424, 313, 211, 169	tetragalloylglucose*
41	19.4	636	635 [M − H]$^-$	617, 545, 483, **465**, 314, 213	313, 249, **169**	trigalloylglucose
42	19.8	786	785 [M − H]$^-$	765, **633**, 483, 423, 301, 276, 241	**301**, 284, 275	HHDP-digalloylglucose
43	19.8	636	635 [M − H]$^-$	617, 483, 466, **423**, 405, 271, 211, 193	**271**, 251, 235, 211, 193, 179, 169	trigalloylglucose
45	20.4	786	785 [M − H]$^-$	765, **633**, 615, 482, 393, 301	483, 447, **301**, 187	HHDP-digalloylglucose
48	22.5	788	787 [M − H]$^-$	635, **617**, 573, 403, 325	573, 529, **465**, 404, 313, 211, 197	tetragalloylglucose
49	23.1	788	787 [M − H]$^-$	**635**, 617, 483, 465, 447	617, **483**, 465, 423, 357, 331, 313, 271, 253, 235, 212, 193	tetragalloylglucose
57	26.2	940	939 [M − H]$^-$	787, **769**, 617	725, **617**, 601, 573, 465, 431, 387, 295, 260	pentagalloylglucose*
58	27.3	940	939 [M − H]$^-$	**787**	769, **635**, 617, 483, 465, 447, 277	pentagalloylglucose
61	29.6	1092	1091[M − H]$^-$	**939**, 787	787, **769**, 617	hexagalloylglucose*
			Flavan-3-ols and Condensed Tannins			
34	17.3	730	729 [M − H]$^-$	641, **577**, 407, 299, 211	411, **289**	galloyl procyanidin dimer*
36	17.6	578	577 [M − H]$^-$	559, 515, **425**, 407, 289, 228, 161	**407**, 299, 257	procyanidin dimer
46	20.4	290	289 [M − H]$^-$	**245**, 205, 188, 179, 165, 137, 126, 110	227, **203**, 191, 161, 123	epicatechin#
53	24.2	442	441 [M − H]$^-$	332, **289**, 169, 125	271, **245**, 203, 165, 143	epicatechin gallate*#
60	28.6	594	593 [M − H]$^-$	**441**, 321, 289, 169	397, 332, **289**, 169	(epi)catechin digallate*
			Ellagic Acid and Conjugates			
63	31.6	434	433 [M − H]$^-$	**301**	**300**, 259, 228, 213, 201, 185	ellagic acid xyloside
67	34.0	302	301 [M − H]$^-$	284, **257**, 229, 185		ellagic acid#
			Flavonols			
68	35.6	448	447 [M − H]$^-$	**301**, 273, 151	271, 255, **179**, 164, 151, 121, 107	quercetin 3-*O*-β-rhamnoside#

[a] Ions in boldface indicate the most intense product ion (100% relative intensity). Compounds followed by an asterisk were identified for the first time in muscadine grapes. Compounds followed by a superscript number sign were identified using pure standards. All other compounds were tentatively identified on the basis of mass data.

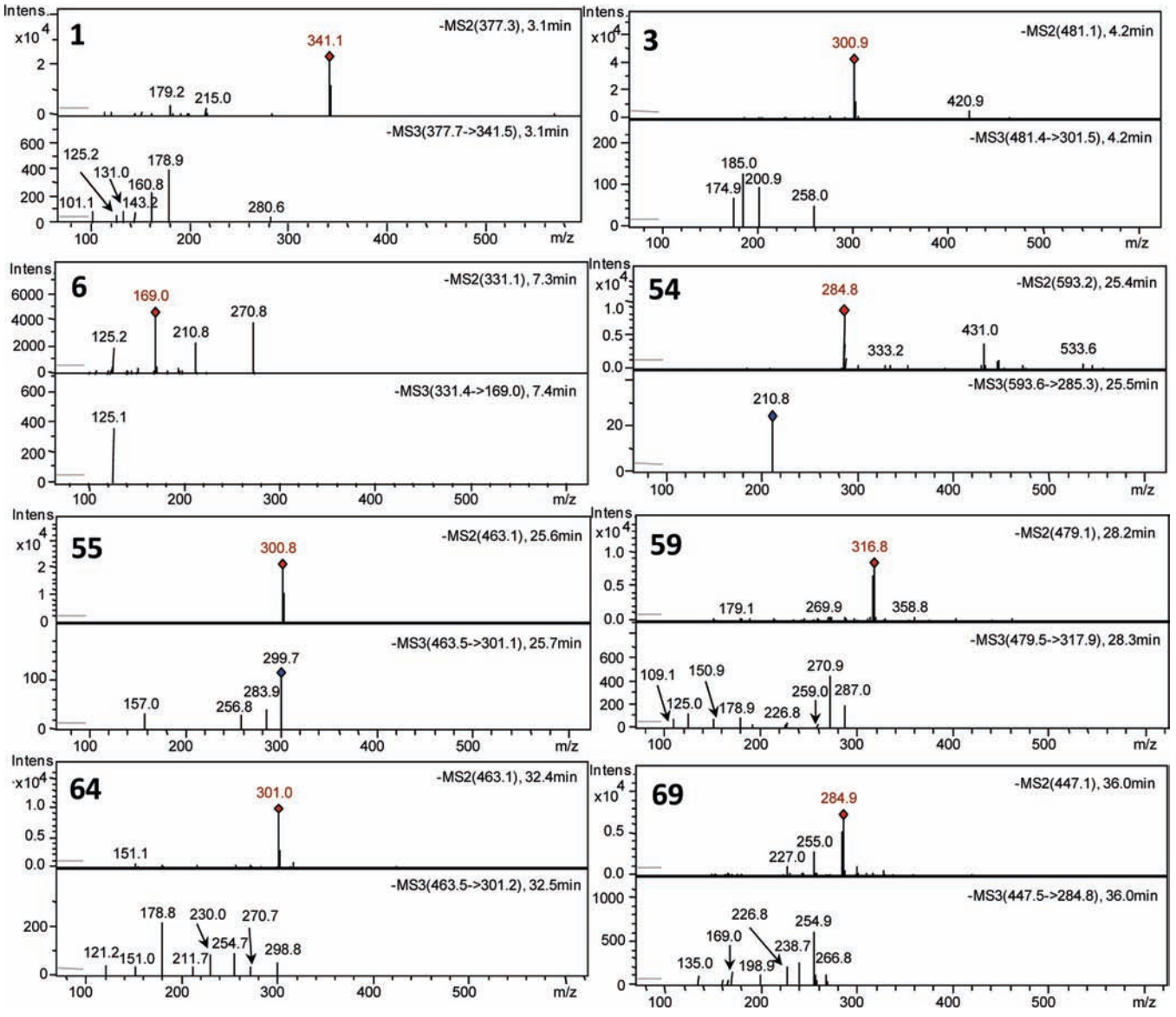


Figure 2. Negative ion electrospray product ion mass spectra (MS^2 and MS^3) of phenolic compounds identified for the first time in muscadine (cv. Noble) pulp, skin, and seeds. The bold numbers in product ion spectra correspond to the compound numbers in the tables.

dissociated to give ion at *m*/*z* 179 by losing a hexose sugar and was tentatively identified as caffeic acid hexoside. Similar MS fragmentation data was observed in previous studies (*24*, *25*).

Hydroxybenzoic Acid. The identification of gallic acid (compound **8**, **Table 4**) was confirmed by the same retention time and MS data of the pure standard which gave an *m*/*z* 169 $[M - H]^-$ ion that dissociated to form an *m*/*z* 125 ion via loss of CO_2. Gallic acid has been previously identified in muscadine grapes (*12*).

Hydrolyzable Tannins. Hydrolyzable tannins are categorized into gallotannins and ellagitannins. Gallotannins consist of a glucose molecule in which hydroxyl groups are partly or completely substituted with galloyl groups, and ellagitannins are esters of the hexahydroxydiphenoyl (HHDP) group consisting of a polyol core (glucose or quinic acid). Additionally, galloyl residues may be attached to the glucose core via *m*-depside bonds (*26*−*29*). On the basis of MS data, the main fragmentation pattern from gallotannins involved the loss of one or more galloyl groups (152 amu) and/or gallic acid (170 amu) from the deprotonated molecule $[M - H]^-$. However, the fragmentation pattern of ellagitannins was less clear than that of gallotannins as ellagitannins display enormous structural variability because of different linkages of HHDP residues with the glucose molecule and their strong tendency to form C−C and C−O−C linkages (*28*, *29*). The presence of the HHDP moiety was confirmed by MS data by the presence of an ion at *m*/*z* 301 from the deprotonated molecule $[M - H]^-$ as reported in previous studies with fruit and plant material (*12*, *24*, *30*−*35*). The presence of a compound with the same molecular weight at different retention times illustrated isomeric forms of that compound. Different isomeric forms of hydrolyzable tannins were observed and have been reported previously in eucalyptus (*34*).

Compound **2** (**Table 4**) and compound **3** (**Table 3**) had an *m*/*z* 481 $[M - H]^-$ ion that fragmented to gave an intense product ion at *m*/*z* 301 $[M - H - 162]^-$ by losing one glucose unit (**Figure 2**). On the basis of the fragmentation pattern and literature data (*34*), these compounds were tentatively identified as isomers of HHDP-glucose. Compounds **7**, **10**, and **16** (**Table 4**) had identical *m*/*z* 483 $[M - H]^-$ ions that fragmented to form ions at *m*/*z* 331 $[M - H - 152]^-$ and *m*/*z* 169 $[M - H - 162]^-$ after sequential removal of the galloyl group and the glucosyl group, respectively. They were tentatively identified as isomers of digalloylglucose on the basis of fragmentation data and previous literature reports (*31*, *34*−*37*).

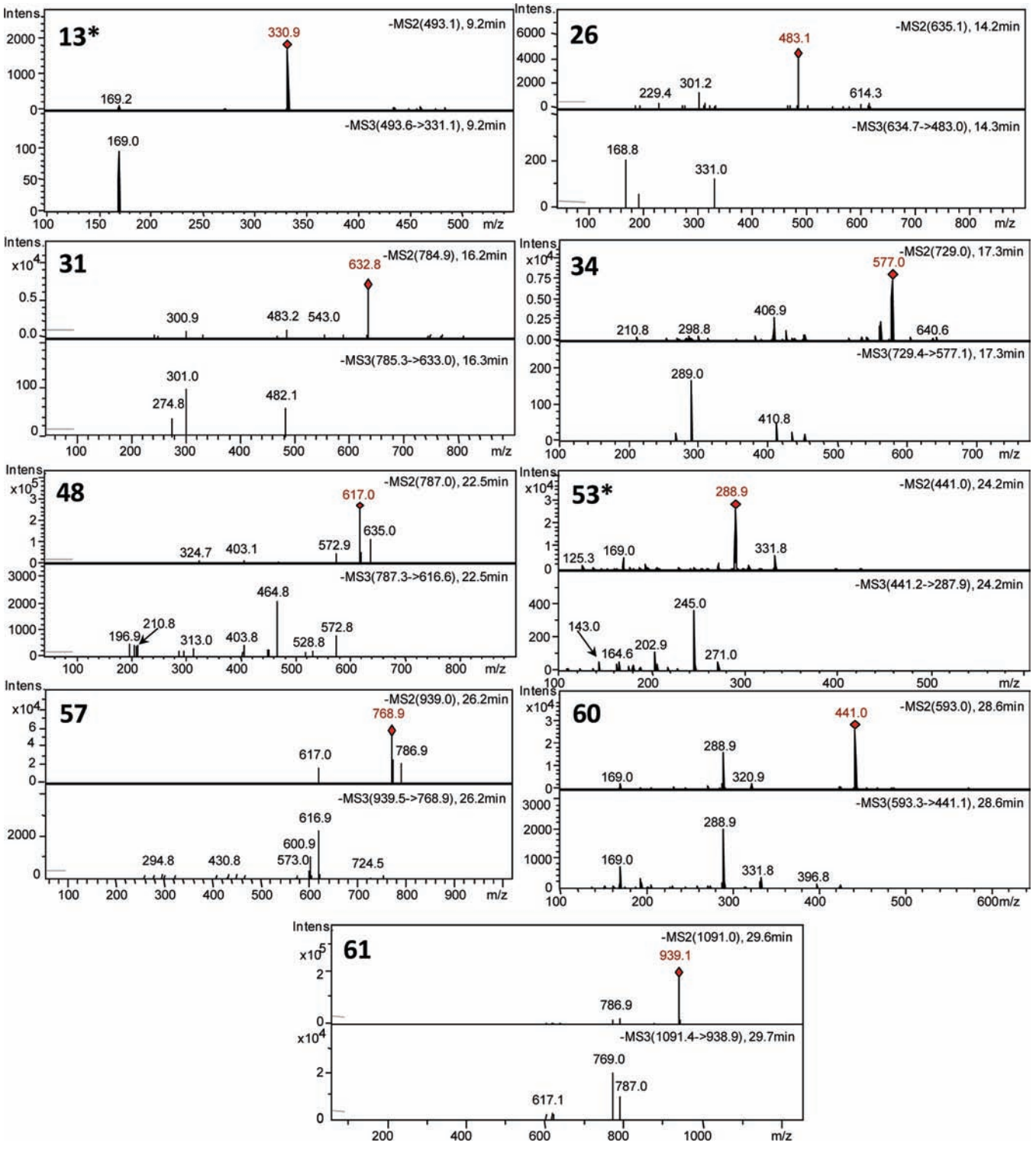


Figure 3. Negative ion electrospray product ion mass spectra (MS^2 and MS^3) of phenolic compounds identified for the first time in muscadine (cv. Noble) seeds. The bold numbers in product ion spectra correspond to the compound numbers in the tables. Compound numbers followed by an asterisk were also identified in the pulp.

Compound **6** (**Tables 2** and **3** and **Figure 2**) gave identical $[M - H]^-$ ions at m/z 331, which yielded a deprotonated gallic acid residue (m/z 169) because of the loss of glucose unit $[M - H - 162]^-$. The gallic acid anion decarboxylates to form a fragment at m/z 125 (169 − 44, loss of CO_2). This compound was tentatively identified as monogalloylglucose (*37*, *38*). Compound **4** (**Table 2**), compound **11** (**Table 3**), and compounds **5**, **9**, and **19** (**Table 4**) eluted at different times but gave $[M - H]^-$ and product ions identical to those of compound **6**. These compounds are tentatively identified as isomers of monogalloylglucose, where gallic acid is attached to a different hydroxyl group of the glucose. Compound **13** (**Tables 2** and **4** and Figure **3**) was tentatively identified as monogalloyldiglucose with an m/z 493 $[M - H]^-$ ion fragmenting to yield ions at m/z 331 and 169 after sequential removal of two glucosyl groups (162 amu). Similar observations were also reported for Longan seeds (*38*). Compound **22** (**Table 3**) had an m/z 625 $[M - H]^-$ ion that dissociated to give an intense MS^2 m/z 463 $[M - H - 162]^-$ ion indicating the loss of a glucose unit. The major ion in MS^3 was at m/z

301 $[M - H - 162 - 162]^-$, suggesting the loss of another glucose unit. On the basis of fragmentation data, this compound was tentatively identified as HHDP-diglucoside (*12*).

Compound **12** (**Table 4**) had an $[M - H]^-$ ion at *m/z* 633 and a major MS^2 fragment ion at *m/z* 481, indicating the presence of HHDP-glucose via the loss of a galloyl unit $[M - H - 152]^-$ and a minor fragment at *m/z* 301. The loss of a galloyl unit suggested that galloyl units were not directly linked to the glucose core but were attached via an *m*-depside bond; thus, the compound was identified as galloyl-HHDP-glucose (*30*, *35*). However, compound **15** (**Table 3**) and compounds **14**, **17**, **20**, **23**, **28**, and **30** (**Table 4**) gave identical deprotonated ions at *m/z* 633 $[M - H]^-$, but the intense MS^2 fragment was at *m/z* 301 $[M - H - 331]^-$ instead of *m/z* 481, indicating the loss of galloylglucose. The fragmentation data suggest that the galloyl unit was directly linked to the glucose core; thus, the compounds were tentatively identified as isomeric forms of HHDP-galloylglucose. Compounds **21**, **24**, **31** (**Figure 3**), **37**, **42**, and **45** (**Table 4**) were tentatively identified as isomeric forms of HHDP-digalloylglucose. The deprotonated molecule with an *m/z* 785 $[M - H]^-$ ion fragmented to give a major ion at *m/z* 633 $[M - H - 152]^-$ indicating the loss of a galloyl group and minor *m/z* 483 $[M - H - 301]^-$ and *m/z* 301 $[M - H - 483]^-$ ions showing the presence of the HHDP moiety and the loss of digalloylglucose (*12*, *31*, *35*).

Compounds **25**, **26** (**Figure 3**), **32**, **35**, **41**, and **43** (**Table 4**) had $[M - H]^-$ ions at *m/z* 635. The major ions in MS^2 were at *m/z* 483 $[M - H - 152]^-$, 465 $[M - H - 170]^-$, and 423 $[M - H - 212]^-$, indicating the loss of a galloyl group, the loss of gallic acid, and the loss of another galloyl group along with cross ring fragmentation of glucose (*39*), respectively. However, MS^3 yielded fragments at *m/z* 331 $[M - H - 152 - 152]^-$, 313 $[M - H - 152 - 152 - 18]^-$, 271 $[M - H - 212 - 152]^-$, and 169, indicating successive losses of galloyl groups. Some other minor fragments were also observed via MS^3. The different retention time and fragmentation pattern suggest the presence of isomeric forms of a given molecule. These compounds were tentatively identified as isomers of trigalloylglucose (*31*, *34*–*36*). Compounds **47**, **51**, and **52** (**Table 3**) were tentatively identified as ellagitannins, yielding deprotonated ions at *m/z* 813, 831, and 817. The presence of HHDP was supported by the formation of an *m/z* 301 ion. However, structural elucidation of these compounds was not conducted due to the lack of complete fragmentation data. Ellagitannins have been previously identified in muscadine grapes (*12*).

Compounds **40**, **48** (**Figure 3**), and **49** (**Table 4**) were tentatively identified as isomers of tetragalloylglucose which dissociated to give identical *m/z* 787 $[M - H]^-$ ions. The fragmentation of the deprotonated ion in MS^1 and MS^2 yielded ions at *m/z* 635 $[M - H - 152]^-$, 617 $[M - H - 152 - 18]^-$, 483 $[M - H - 152 - 152]^-$, 465 $[M - H - 152 - 152 - 18]^-$, 313 $[M - H - 152 - 152 - 18 - 152]^-$, and 169 $[M - H - 152 - 152 - 18 - 152 - 144]^-$, indicating consecutive losses of galloyl groups and water molecules, and finally a loss of glucose from the dehydrated galloylglucose molecule to give deprotonated gallic acid. These findings were confirmed by previous literature reports (*31*, *34*, *35*). Compounds **57** (**Figure 3**) and **58** (**Table 4**) gave identical *m/z* 939 $[M - H]^-$ ions dissociating to yield ions at *m/z* 787 $[M - H - 152]^-$, 769 $[M - H - 152 - 18]^-$, 635 $[M - H - 152 - 18 - 134]^-$, 617 $[M - H - 152 - 152 - 18]^-$, 483 $[M - H - 152 - 152 - 18 - 152]^-$, and 465 $[M - H - 152 - 152 - 18 - 152 - 18]^-$, suggesting the loss of galloyl groups and water molecules. Because of the lack of complete structural elucidation by fragmentation data in MS^1 and MS^2, these compounds were tentatively identified as isomers of pentagalloylglucose (*35*).

Compound **61** (**Table 4** and **Figure 3**) was tentatively identified as hexagalloylglucose (*35*) with an *m/z* 1091 $[M - H]^-$ ion that fragmented to give *m/z* 939 $[M - H - 152]^-$ and 787 $[M - H - 152 - 152]^-$ ions in MS^2 indicating the loss of galloyl groups and the presence of pentagalloyl- and tetragalloylglucose residues. The MS^3 fragments were at *m/z* 787 $[M - H - 152 - 152]^-$, 769 $[M - H - 152 - 152 - 18]^-$, and 617 $[M - H - 152 - 152 - 18 - 152]^-$, indicating the loss of galloyl groups and water molecules. Similar results were reported by Soong et al. (*38*).

Anthocyanins. Six different anthocyanins were identified in muscadine grape skin [compounds **27**, **29**, **33**, **38**, **39**, and **44** (**Table 3**)]. The anthocyanins coeluted and represented only three peaks in the chromatogram, but they had different retention times. Therefore, the peak numbers were marked according to the retention times of individual anthocyanins (Figure S2C of the Supporting Information). Although previous studies have identified and quantified the anthocyanins in muscadine grapes (*9*, *40*), most of the identification and quantification was conducted after hydrolysis (*9*, *11*) which does not justify the structure of anthocyanin diglucosides. In this study, the fragmentation pattern of anthocyanins from MS^2 and MS^3 is provided. A similar fragmentation pattern was observed for all anthocyanins, indicating the loss of glucose residues and formation of aglycone. Anthocyanins in muscadine grapes have been reported to exist in 3,5-diglucoside forms (*40*–*42*).

Compound **27** with an *m/z* 627 $[M]^+$ ion fragmented to two product ions via MS^2 at *m/z* 465 $[M - 162]^+$ and 303 $[M - 162 - 162]^+$, corresponding to delphinidin glucoside and delphindin, respectively. Therefore, this compound was tentatively identified as delphinidin 3,5-diglucoside. Compound **29** had an *m/z* 611 $[M]^+$ ion that fragmented to yield two product ions at *m/z* 449 $[M - 162]^+$ and 287 (cyanidin) $[M - 162 - 162]^+$, indicating the compound to be cyanidin 3,5-diglucoside. Compound **33** was tentatively identified as petunidin 3,5-diglucoside. The molecular ion at *m/z* 641 $[M]^+$ fragmented to yield two product ions via MS^2 at *m/z* 479 $[M - 162]^+$ and 317 (petunidin) $[M - 162 - 162]^+$, indicating two glucose molecules attached to petunidin. Compounds **38** and **39** co-eluted with the same retention time, but the mass spectrum of the peaks suggested two molecular ions at *m/z* 595 $[M]^+$ and 625 $[M]^+$. The MS^2 spectrum of the molecular ion at *m/z* 595 (compound **38**) fragmented into two product ions at *m/z* 433 $[M - 162]^+$ and 271 $[M - 162 - 162]^+$, which corresponded to pelargonidin glucoside and pelargonidin, respectively. Compound **38** was tentatively identified as pelargonidin 3,5-diglucoside. However, this pigment was not identified in other black varieties studied (Magoon, Southland, and Alachua). Similarly, the molecular ion at *m/z* 625 (compound **39**) had two product ions at *m/z* 463 $[M - 162]^+$ and 301 $[M - 162 - 162]^+$, which corresponded to peonidin glucoside and peonidin, respectively. This compound was tentatively identified as peonidin 3,5-diglucoside. Compound **44** had a molecular ion at *m/z* 655 $[M]^+$ and fragment ions at *m/z* 493 $[M - 162]^+$ and 331 $[M - 162 - 162]^+$. On the basis of mass fragmentation, this compound was tentatively identified as malvidin 3,5-diglucoside. We were able to confirm the presence of pelargonidin 3,5-diglucoside in muscadine grapes as reported in a previous study (*9*).

Flavan-3-ols and Condensed Tannins. The condensed tannins were identified only in muscadine seeds compared to skin and pulp. Both galloylated and nongalloylated flavan-3-ols and condensed tannins were identified (**Tables 2**–**4**). Compound **18** (**Table 3**) had a deprotonated ion at *m/z* 305 $[M - H]^-$ and was identified as gallocatechin. Its identity was confirmed because it had the same retention time as the standard and via the formation of MS^2 fragment ions at *m/z* 285, 263, 219, 179, 165, and 125 (*12*, *43*). Compound **34** (**Table 4** and **Figure 3**) with an *m/z* 729 $[M - H]^-$ ion generated a main MS^2 fragment ion at *m/z* 577 $[M - H - 152]^-$ and an MS^3 fragment ion at *m/z* 289 $[M - H - 152 - 288]^-$,

corresponding to the loss of a galloyl group and an (epi)catechin gallate moiety, respectively. On the basis of mass spectral data, this compound was tentatively identified as the galloyl procyanidin dimer (*43*, *44*). Compound **36** (**Table 4**) was tentatively identified as the procyanidin dimer with its *m*/*z* 577 $[M - H]^-$ ion dissociating to yield ions at *m*/*z* 425 $[M - H - 152]^-$ and 289 $[M - H - 288]^-$ in MS^2, indicating the characteristic loss of 152 amu due to retro-Diels–Alder (RDA) fission (*43*) and loss of an (epi)catechin molecule, respectively. Mass spectral data from MS^3 showed further dissociation of flavanol rings. Compound **46** (**Tables 2** and **4**) with identical $[M - H]^-$ ions at *m*/*z* 289 generated the major MS^2 ions at *m*/*z* 245 (loss of CO_2) and minor ions at *m*/*z* 205 (cleavage of the A ring of flavan-3-ol) and 137 (RDA fission). The major ion in MS^3 was at *m*/*z* 203 (cleavage of the A ring of flavan-3-ol). Compared with the standard, this compound was identified as epicatechin (*16*, *24*, *30*). Compound **53** (**Tables 2** and **4** and **Figure 3**) dissociated at *m*/*z* 441 $[M - H]^-$ to yield product ions in MS^2 at *m*/*z* 289, 169, and 125 corresponding to the deprotonated ion of (epi)catechin and gallic acid and decarboxylated gallic acid, respectively. The major fragment ion in MS^3 was at *m*/*z* 245, indicating decarboxylation of epicatechin, and minor fragments at *m*/*z* 205 and 137 suggesting the characteristic fragmentation pattern of (epi)catechin (*30*, *43*). On the basis of it having the same retention time as the standard and mass spectral data, this compound was identified as epicatechin gallate. Compound **60** (**Table 4** and **Figure 3**) gave an $[M - H]^-$ ion at *m*/*z* 593, which yielded major ions at *m*/*z* 441 $[M - H - 152]^-$ in MS^2 spectra and *m*/*z* 289 $[M - H - 152 - 152]^-$ in MS^3 spectra, corresponding to the loss of successive galloyl groups from (epi)catechin. On the basis of the mass spectral data, this compound was tentatively identified as (epi)catechin digallate (*45*).

Ellagic Acid and Conjugates. Ellagic acid has been identified and quantified in muscadine grapes in previous studies (*8*, *12*). In this study, we identified ellagic acid hexoside in Noble pulp and skin for the first time (**Tables 2** and **3**). Compound **55** (**Tables 2** and **3** and **Figure 2**) had an identical $[M - H]^-$ ion at *m*/*z* 463 which yielded a major ion at *m*/*z* 301 $[M - H - 162]^-$ and minor ions at *m*/*z* 284, 257, and 229 characteristic of ellagic acid fragmentation (*16*, *30*). The loss of 162 amu corresponded to the loss of a hexose sugar, and on the basis of the fragmentation pattern, this compound was tentatively identified as ellagic acid hexoside (*31*, *46*). Compound **63** (**Tables 2–4**) was tentatively identified as ellagic acid xyloside with its $[M - H]^-$ ion at *m*/*z* 433 dissociating to form a major ion at *m*/*z* 301 via the loss of xylose (132 amu) and minor ions at *m*/*z* 284, 257, and 185 indicating the presence of ellagic acid (*12*, *32*, *38*). The MS spectra of compound **65** (**Tables 2** and **3**) gave identical $[M - H]^-$ ions at *m*/*z* 447 that dissociated to yield a major ion at *m*/*z* 301 $[M - H - 146]^-$ and minor ions at *m*/*z* 257, 229, and 185 corresponding to the loss of rhamnose and characteristic fragmentation pattern of ellagic acid. Therefore, this compound was tentatively identified as ellagic acid rhamnoside. Compound **67** (**Tables 2–4**) was identified as free ellagic acid on the basis of the characteristic fragmentation pattern, the standard, and previously published data (*12*, *16*, *24*, *30*, *38*, *46*).

Flavonols. The flavonols identified in muscadine grapes were glycosides of quercetin, kaempferol, and myricetin (*12*). Myricetin hexoside, kaempferol hexoside, quercetin glucoside, and kaempferol rutinoside were identified for the first time (**Tables 2** and **3**). Compound **54** (**Tables 2** and **3** and **Figure 2**) had an identical $[M - H]^-$ ion at *m*/*z* 593 which fragmented to produce product ions at *m*/*z* 447 $[M - H - 146]^-$ and 285 $[M - H - 146 - 162]^-$, indicating the loss of a rhamnosyl group and a hexosyl-rhamnosyl group, respectively. The fragment ion at *m*/*z* 285 corresponded to aglycone of kaempferol; therefore, this compound was tentatively identified as kaempferol rutinoside (*43*). Compound **59** (**Table 3** and **Figure 2**) with a deprotonated ion at *m*/*z* 479 $[M - H]^-$ gave product ions at *m*/*z* 317 $[M - H - 162]^-$ and minor ions at *m*/*z* 271, 179, and 151, suggesting the loss of hexose and producing aglycone myricetin. On the basis of the mass spectral data and previously published data (*43*, *47*), this compound was tentatively identified as myricetin hexoside. Compound **62** (**Tables 2** and **3**) was tentatively identified as myricetin rhamnoside (464 amu) on the basis of MS data that produced the major fragment of aglycone myricetin (*m*/*z* 317) via the loss of rhamnose (146 amu) (*12*, *25*). Compound **64** (**Table 3** and **Figure 2**) had an *m*/*z* 463 $[M - H]^-$ ion, dissociating to yield fragment ions at *m*/*z* 301 $[M - H - 162]^-$, 271, 179, and 151, characteristic of quercetin fragmentation. This compound was identified as quercetin 3-*O*-*β*-glucoside on the basis of mass spectral data and the standard (*16*, *47*, *48*). Compound **68** (**Tables 2–4**) was identified as quercetin 3-*O*-*β*-rhamnoside on the basis of standard and mass fragmentation which produced an *m*/*z* 447 $[M - H]^-$ ion and dissociated to give ions at *m*/*z* 301 $[M - H - 146]^-$ and *m*/*z* 179 and 151, corresponding to the loss of rhamnose and fragmentation of quercetin, respectively (*12*, *38*, *48*). Compound **69** (**Tables 2** and **3** and **Figure 2**) gave a deprotonated ion at *m*/*z* 447 which further fragmented to produce a major ion at *m*/*z* 285 $[M - H - 162]^-$ and minor ions at *m*/*z* 255 and 227; the compound was tentatively identified as kaempferol hexoside (*30*, *48*). Compound **70** (**Tables 2** and **3**) had an *m*/*z* 431 $[M - H]^-$ ion, which fragmented to yield a major ion at *m*/*z* 285 $[M - H - 146]^-$ in MS^2. On the basis of mass spectral data and a previous study of muscadine grapes (*12*), this compound was tentatively identified as kaempferol rhamnoside.

Stilbenes. Compounds **50** and **56** (**Table 2**) had *m*/*z* 425 $[M - H]^-$ ions, due to formation of the chloride adduct. The ion at *m*/*z* 425 $[M + Cl^-]$ dissociated to yield two product ions, one at *m*/*z* 389 $[M - H]^-$ and the other at *m*/*z* 227 $[M - H - 162]^-$, corresponding to the loss of chloride ion and glucose, respectively. Similarly, compound **66** (**Table 3**) had an *m*/*z* 389 $[M - H]^-$ ion. The MS^2 spectrum of the deprotonated ion at *m*/*z* 389 produced a product ion at *m*/*z* 227 $[M - H - 162]^-$ resulting from the loss of a glucose unit. On the basis of mass spectral data and the standard, compound **56** was identified as *trans*-resveratrol 3-*O*-*β*-glucoside. The other two compounds (**50** and **66**) having the same mass spectral data were tentatively identified as isomeric forms of resveratrol glucoside (*44*, *49*).

In addition to similar compounds found in different grape cultivars, a few compounds that were identified in other cultivars include catechin and its derivatives in the seeds, and protocatechuic acid hexoside and epigallocatechin in the skin (data not shown).

Our results indicate that muscadine seeds have high phenolic content and antioxidant capacity compared to skin and pulp. The high antioxidant capacity and total phenolic content of the muscadine seeds make them a potentially significant source of compounds with nutraceutical properties. Additionally, the results confirmed that HPLC-ESI-MS^n, operated under both positive and negative ionization, is a valuable tool for the identification of a wide array of known phenolic compounds as well as for the preliminary identification of novel compounds. This method allows simultaneous identification of various phenolic compounds (phenolic acids, anthocyanins, flavonols, flavan-3-ols and condensed tannins, hydrolyzable tannins, and stilbenes) under similar chromatographic conditions. The prominent class of phenolic compounds in muscadine skin and pulp is the flavonol group, compared to seeds in which the majority of compounds belong to the hydrolyzable and condensed tannin category. The phenolic

compounds from the skin and seeds are extracted into the wine and juice and are important quality components that contribute to the color and taste of these products. Thus, the structural elucidation of phenolic compounds in muscadine grapes could provide an improved understanding of color and flavor changes occurring in muscadine wine and juice upon storage.

Supporting Information Available: HPLC-DAD chromatograms of muscadine (cv. Noble) pulp (Figure S1), HPLC-DAD chromatograms of muscadine (cv. Noble) skin (Figure S2), and HPLC-DAD chromatograms of muscadine (cv. Noble) seeds (Figure S3). This material is available free of charge via the Internet at http://pubs.acs.org.

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