Screening for Annonaceous Acetogenins in Bioactive Plant Extracts by Liquid Chromatography/Mass Spectrometry

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The Annonaceous acetogenins are amenable to analysis by liquid chromatography/mass spectrometry (LC/MS) using the electrospray positive-ion mode. Under conditions of atmospheric pressure in-source collision-induced dissociation (APICID), the acetogenins reproducibly provided characteristic ion patterns and fragment ions. Accordingly, the presence of these biologically interesting acetogenins and other derivatives in plant extracts and chromatographic fractions can be readily detected by analyzing selected ion chromatograms. LC/MS screening of a bioactive crude methanol-soluble fraction of *Rollinia mucosa* detected the presence of some 40 known acetogenins in this plant extract, in addition to four new acetogenins of diverse structure. This rapid and relatively uncomplicated selected ionization procedure should also prove suitable for the screening of many other structural families of natural products.

In the isolation and purification of bioactive natural products, remaining major challenges are the efficient detection of a desired class of compounds within a mixture and how to monitor purification. While bioactivity-directed fractionation is effective, it is usually labor intensive and expensive and consumes often-scarce sample. In practice, the search of bioactive fractions for specific, desired active compounds with predictable structures, representing a particular class of phytochemicals, has employed other detection methods, e.g., UV, TLC, and ^{1}H-NMR.[1,2]

However, liquid chromatography/mass spectrometry (LC/MS) has recently attracted attention as a convenient method for analyzing different classes of metabolites, particularly those detected only with difficulty by UV or other spectroscopic methods.[3−5] Development of the atmospheric electrospray ion (ESI) mode has greatly increased the sensitivity of LC/MS and has dramatically decreased the amount of test sample required for structural analysis.[6,7] The combination of fine separation by HPLC and high sensitivity of the mass spectrometer allows for LC/MS detection of specific natural product families, even in crude extracts. LC/MS further allows for target compounds to be screened rapidly and can supply structural information that often is not readily available. Since the separation process in LC/MS parallels that of large-scale isolation procedures, it is possible to track the separation of target compounds. This broader application of LC/MS thus allows for recognition of the presence of known compounds in mixtures and provides evidence of novel natural products that are not readily detectable by other analytical methods.

The Annonaceous acetogenins are bioactive natural products that act as potent inhibitors of mitochondrial NADH:ubiquinone oxidoreductase and of the ubiquinone-linked NADH oxidase peculiar to the plasma membranes of tumor cells.[8−11] These compounds have very weak UV absorbance in the short wavelength range (*ca.* 220 nm) and cannot be detected readily by UV in the presence of large amounts of impurities. This makes their isolation and purification very difficult. However, the acetogenins show high sensitivity to analysis by LC/MS using the electrospray positive-ion mode [LC/(+)-ESI-MS].

In this paper, we report a novel, rapid screening procedure for acetogenins in crude plant extracts using LC/MS. This facile procedure has proven useful for the detection of both previously reported and new compounds in the search for novel bioactive acetogenins. It also allows for a focused identification of selected known acetogenins that are targeted for reisolation and further biological evaluation.

Results and Discussion

LC/(+)ESI-MS experiments were applied initially to nine pure acetogenins (Table 1) isolated from *Rollinia mucosa*. Using 1% formic acid and methanol as the HPLC mobile phase, the protonated molecular ions of the Annonaceous acetogenins were either very weak or undetectable, and the dominant ions were the sodium adduct molecular ions $[M + Na]^+$ (+23 amu), which produced few fragment ions by daughter ion scanning (MS/MS). When 1% formic acid was replaced with dilute ammonium acetate buffer (0.01 M, pH 4), partial sodium adduct cations were converted to ammonium adduct cations $[M + NH_4]^+$ (+18 amu). The LC/(+)-ESI-MS spectrum of bullatalicin is presented as an example in Figure 1. The spectrum exhibited the characteristic molecular adduct ions at *m/z* 639 ($[M + H]^+$), 656 ($[M + NH_4]^+$), and 661 ($[M + Na]^+$).

LC/MS experiments were then conducted using atmospheric pressure in-source collision-induced dissociation conditions [LC/(+)ESI-APICID-MS]. The acetogenins were found to produce the most intense protonated

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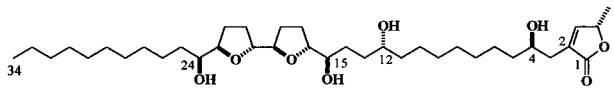
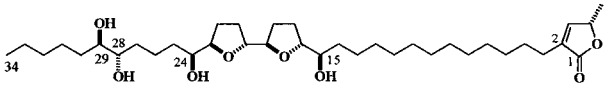
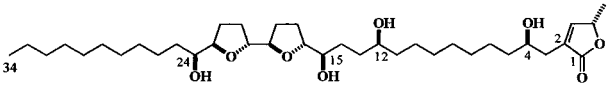
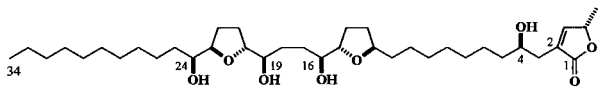
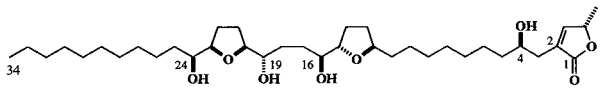
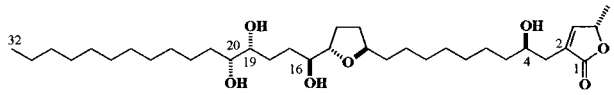
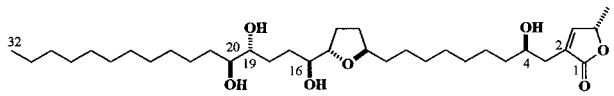
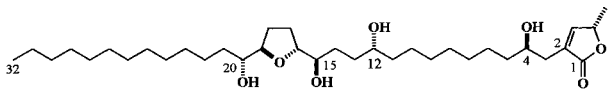
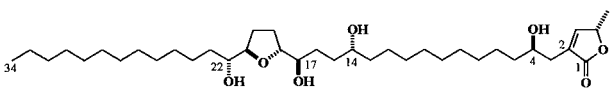
Table 1. Structures, Molecular Weights, and HPLC Retention Times of Nine Acetogenins from *Rollinia mucosa*

Compound	Structure	mol wt	R_t (min)
12-Hydroxy-bullatacin A		638	37.8
Rollitacin		638	37.8
12-Hydroxy-bullatacin B		638	38.3
Bullatalicin		638	39.5
Sylvaticin		638	42.1
Muricatetrocin C		596	42.5
Muricatetrocin B		596	44.1
Javoricin		596	44.7
Rollinecin A		624	47.2

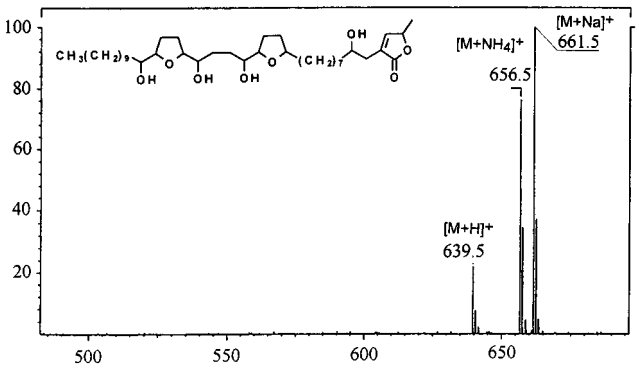


Figure 1. LC/(+)ESI-MS spectrum of bullatalicin (mol wt: 638 Da).

molecular ions under a collision energy of *ca.* 20 eV. In addition, fragment ions derived from successive losses of H_2O (three to five molecules), representative of the multihydroxylated structures of the acetogenins, also were present. Thus, the presence of the acetogenins could be detected by their characteristic ion patterns, $[M + Na]^+$, $[M + H]^+$, $[M + H - H_2O]^+$, $[M + H - 2H_2O]^+$, $[M + H - 3H_2O]^+$, and $[M + H - 4H_2O]^+$ (Figure 2B). LC/(+)ESI-APICID-MS/MS was applied to intense $[M + H]^+$ ions. Similar to the results of LC/(+)ESI-APICID-MS, the predominant fragment ions observed were sequentially dehydrated molecular ions.

When screening for desired components in a crude extract by LC/MS alone, a full ion scan offers little help since high levels of impurities cause strong interference and prohibit the determination of desired ions. To detect desired compounds in a crude extract by LC/MS, an ion pattern consisting of a group of correlated ions is essential. Usually, for pure compounds, MS/MS produces direct daughter ions and provides convincing structural evidence. However, to conduct LC/MS/MS experiments, the molecular weight of each component to be dissociated must be previously known. This is not possible for a chromatographic fraction containing all unknown compounds. Even in cases where known compounds are being sought, if an extract or a crude fraction contains several desired components, multiple runs are usually needed. The APICID-MS method solves these problems. A single LC/ESI-APICID-MS scan of a crude extract can provide the parent as well as daughter ions derived from all of the desired compounds. Although large amounts of ions from impuri-

Table 2. Expected Molecular Weights of Different Structural Types of Annonaceous Acetogenins

	bis-THF (or THP)[a]					mono-THF (or THP)[b]			
	C_{37}		C_{35}			C_{37}		C_{35}	
no. of OH groups[c]	w/o[d]	w/[e]	w/o[d]	w/[e]	no. of OH groups[c]	w/o[d]	w/[e]	w/o[d]	w/[e]
5	654	652	626	624	6	656	654	628	626
4	638	636	610	608	5	640	638	612	610
3	622	620	594	592	4	624	622	596	594
2	606	604	578	576	3	608	606	580	578
					2	592	590	564	562

[a] Bis-tetrahydrofuran ring (THF) or tetrohydropyran ring (THP). [b] Mono-tetrahydrofuran ring (THF) or tetrahydropyran ring (THP). [c] Number of hydroxyl groups present in the molecule. [d] Possession of no double bonds. [e] Possession of one double bond.

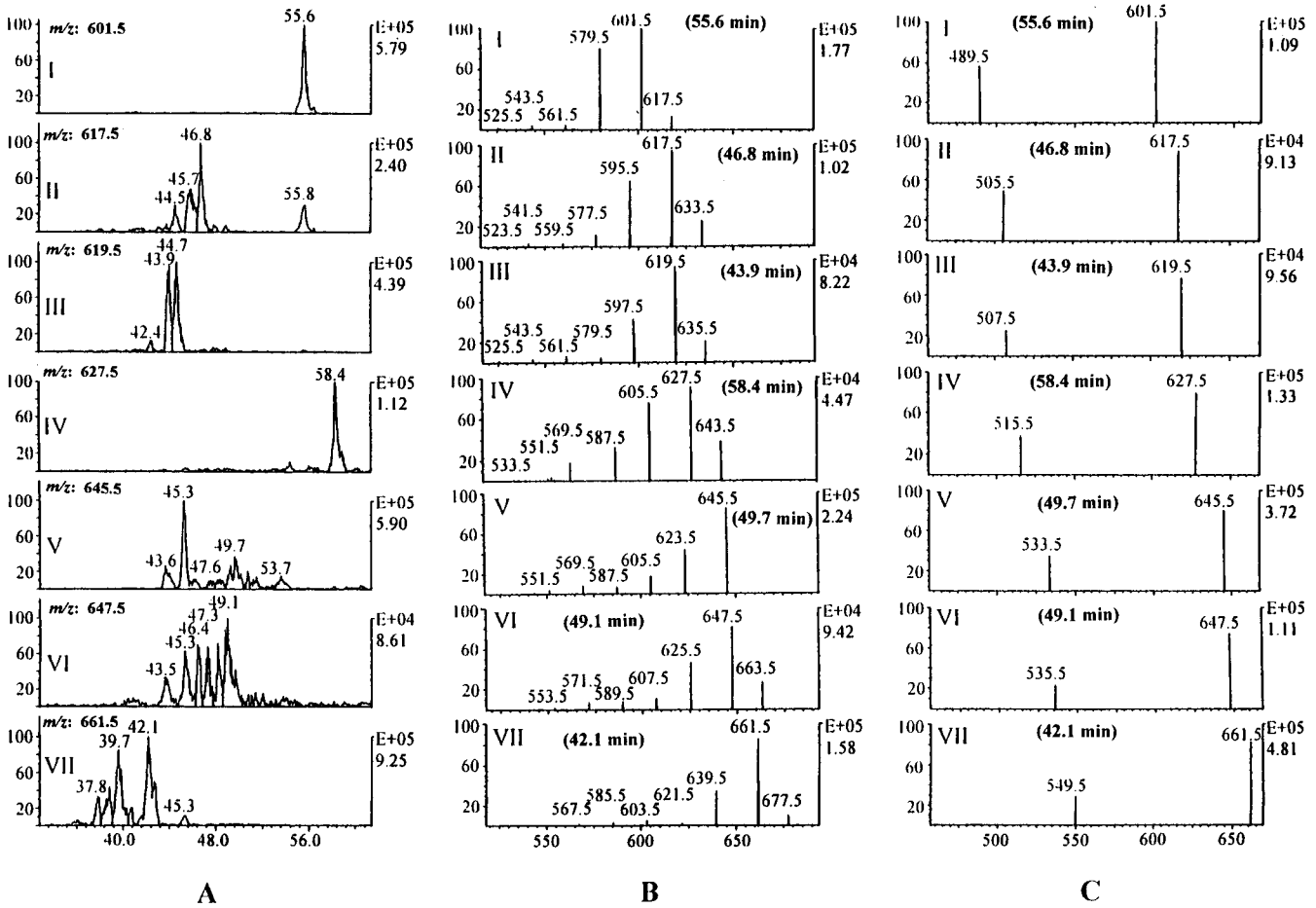


Figure 2. (A) Selected ion chromatograms of $[M + Na]^+$ at *m/z* 601 (I), 617 (II), 619 (III), 627 (IV), 645 (V), 647 (VI), and 661 (VII) of acetogenins present in a methanol-soluble fraction of *Rollinia mucosa*. (B) The representative LC/(+)ESI-APICID-MS spectra (retention times are labeled on each spectrum) of $[M + Na]^+$ at *m/z* 601 (I), 617 (II), 619 (III), 627 (IV), 645 (V), 647 (VI), and 661 (VII). (C) The representative LC/(+)ESI-MS/MS spectra (retention times are labeled on each spectrum) of $[M + Na]^+$ at *m/z* 601 (I), 617 (II), 619 (III), 627 (IV), 645 (V), 647 (VI), and 661 (VII).

Table 3. $[M + Na]^+$ or $[M + H]^+$ Ions and Prominent Fragment Ions of Acetogenins Present in a Methanol-Soluble Fraction of *Rollinia mucosa*

mol	$[M + Na]^+$	$[M + Na - 112]^+$	$[M + H]^+$	$[M + H - H_2O]^+$	$[M + H - 2H_2O]^+$	$[M + H - 3H_2O]^+$	$[M + H - 4H_2O]^+$
578	601	489	579	561	543	525	507
594	617	505	595	577	559	541	523
596	619	507	597	579	561	543	525
604	627	515	605	587	569	551	533
622	645	533	623	605	587	569	551
624	647	535	625	607	589	571	553
638	661	549	639	621	603	585	567

ties were also present, our experimental results showed that selected ion chromatograms, obtained by carefully analyzing total ion chromatograms, could supply structurally informative ion patterns. Thus, LC/(+)ESI-APICID-MS was mainly used in this study.

The reversed-phase HPLC (RP-HPLC) retention times (t_R) of nine pure acetogenins isolated from *R. mucosa* are listed in Table 1. The retention times are consistent with the polarities of the acetogenins: i.e., (1) if the chain lengths are the same, compounds possessing more hydroxyl groups are more polar; (2) if the chain lengths and numbers of hydroxyl groups are the same, the polarity sequence is based on tetrahydrofuran (THF) content: adjacent bis-THF acetogenins > nonadjacent bis-THF acetogenins > mono-THF acetogenins; (3) if other functional groups are the same, C_{35} acetogenins are more polar than C_{37} acetogenins; (4) the shifting of a hydroxyl group from C-4 to another position along the chain increases polarity probably due to a weak hydrogen bond formed by the C-4 hydroxyl proton with the carbonyl of the γ-lactone.[7−10]

An aqueous methanol extract of *R. mucosa*[12] was scanned under the LC/(+)ESI-APICID-MS conditions. All expected molecular weights of the acetogenin constituents were calculated (Table 2), and the $[M + H]^+$ ions were searched using selected ion chromatograms (SICs). While many acetogenins are positional or steric isomers and have the same molecular weights,[8−11] such compounds can usually be resolved by RP-HPLC. Therefore, the SIC of each individual $[M + H]^+$ ion exhibits multiple ion peaks (Figure 2A). Those ions that showed peaks in the SICs were further evaluated by acetogenin ion pattern comparisons (Figures 3−5). For example, the ion pattern of $[M + H]^+$ 639 as illustrated in Figure 3 includes SICs of ions at *m/z* 661 $[M + Na]^+$, 639 $[M + H]^+$, 621 $[M + H - H_2O]^+$, 603 $[M + H - 2H_2O]^+$, 585 $[M + H - 3H_2O]^+$, and 567 $[M + H - 4H_2O]^+$. As discussed previously, all acetogenins produce ions that

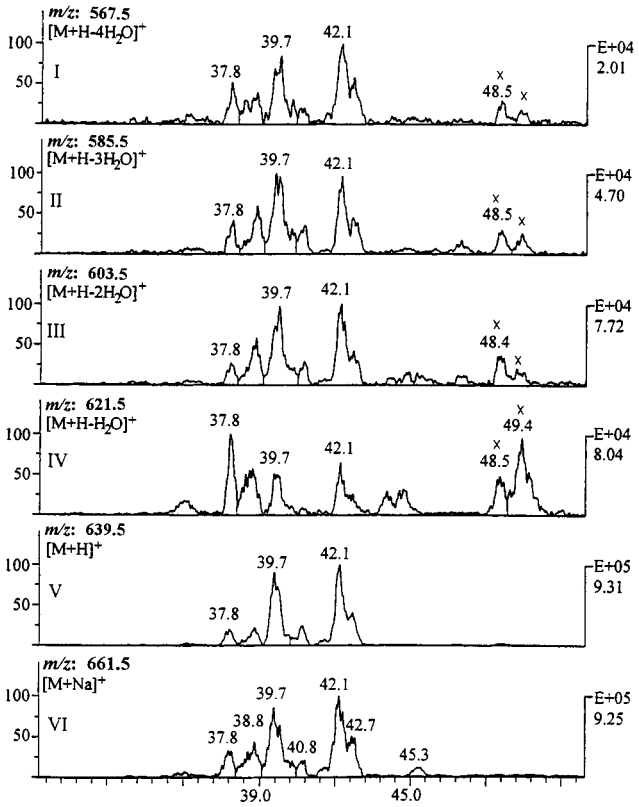


Figure 3. Selected ion chromatograms (SIC) of acetogenins present in a methanol-soluble fraction of *Rollinia mucosa* with a molecular weight of 638 Da. The SIC presented are as follows: *m/z* 661 $[M + Na]^+$ (VI), *m/z* 639 $[M + H]^+$ (V), *m/z* 621 $[M + H - H_2O]^+$ (IV), *m/z* 603 $[M + H - 2H_2O]^+$ (III), *m/z* 585 $[M + H - 3H_2O]^+$ (II), and *m/z* 621 $[M + H - H_2O]^+$ (I). The peaks marked by "x" at t_R = 48.5 and 49.4 min were apparently not produced from acetogenins with this molecular weight.

match this ion pattern. Therefore, only those that exhibited all of these six SICs (t_R 37−43 min) were treated as ion peaks corresponding to the acetogenins having a molecular weight of 638 Da, and the peaks that appeared only in partial SICs, e.g., at t_R 48.5 and 49.4 min (Figure 3, I−IV), were apparently not produced from acetogenins.

In this way, seven protonated molecular ions, *m/z* 639, 625, 623, 605, 597, 595, and 579 (Figures 2A, 3−5), were found to be related to acetogenins. Their representative LC/(+)ESI-APICID-MS spectra are shown in Figure 2B, and the representative LC/(+)ESI-MS/MS spectra of their respective sodium adduct molecular ions are shown in Figure 2C. The *m/z* values of the possible product ions of these acetogenins are summarized in Table 3. It is noted that some *m/z* values repeatedly appear, e.g., *m/z* 605, which can be either the $[M + H]^+$ of acetogenins having a molecular weight of 604 Da or the $[M + H - H_2O]^+$ ion of acetogenins having a molecular weight of 622 Da. However, the former produce a sodium adduct ion at *m/z* 627 $[M + Na]^+$ (Figure 4C, t_R 58.4 min) but the latter do not (Figure 4C, t_R 43.6, 45.3 min). Similar cases can also be observed in Figures 4B, 5A, and 5C. Thus, the actual molecular weights corresponding to the acetogenins present in a particular fraction can be readily determined.

The RP-HPLC retention times shown in Figure 2A support these results. All of the acetogenins with a molecular weight of 638 Da (Figure 2A, VII) eluted earlier than those with a molecular weight of 622 Da (Figure 2A, V). For the bis-THF acetogenins, a molecular weight of 638 Da suggests the presence of four hydroxyl groups, and a molecular weight of 622 Da indicates one less hydroxyl group (Table 2). For example, some possible acetogenins having a molecular weight of 622 are suggested in Figure 6.

Laprévote *et al.* reported that the fast-atom bombardment tandem mass spectrometry (FABMS/MS) of lithium adduct molecular ions ($[M + Li]^+$) of acetogenins yielded an intense daughter ion derived from a loss of 112 amu.[13] We theorized that the sodium adduct ions could exhibit behavior similar to that of the lithium adduct ions. When the LC/(+)ESI-MS/MS spectra of the sodium adduct molecular ions of the pure isolated acetogenins and the crude methanol fraction of *R. mucosa* were analyzed, we observed the facile loss of 112 amu from all of the acetogenins having a C-4 hydroxyl group (Figure 2C) but not from those lacking the hydroxyl group at C-4 (e.g., rollitacin; see Table 1). This suggests a probable ion pathway as illustrated in Figure 7. The LC/MS/MS experiments with sodium adduct molecular ions further confirmed that the molecular ions obtained by analyzing the SIC's resulted from acetogenins.

The presence or absence of a C-4 hydroxyl group is an important differentiating feature among the various acetogenins. To distinguish between these two types of acetogenins, the sodium adduct ions of the acetogenins present in the methanol extract of *R. mucosa* were subjected to the daughter ion scans. For example, the results of the LC/(+)ESI-MS/MS on ions at *m/z* 645 (*m/z* 622 + 23) are displayed in Figure 8. Intense daughter ions at *m/z* 533 (*m/z* 645−112) appeared between t_R 48−55 min and indicated that the acetogenins distributed in this region likely have a C-4 hydroxyl group, since they underwent fragmentation losses of 112 amu. There were only minor signals at *m/z* 533 between t_R 43.6 and 45.3 min; this suggested that such acetogenins probably lack the C-4 hydroxyl group. As previously reviewed, shifting of a hydroxy group from C-4 to another position along the alkyl chain increases polarity because the C-4 hydroxy group forms a weak intramolecular hydrogen bond with the carbonyl of the γ-lactone.[7−10]

Twenty-four acetogenins have been isolated from the methanol fraction of *R. mucosa*.[12,14−19] These compounds are all represented by only four molecular weights (638, 624, 622, and 596 Da). Acetogenins having molecular weights of 578, 594, and 604 Da, which appeared in the SIC search, have not been isolated from this fraction. The daughter ion scans of fragments at *m/z* 601 ($[578 + Na]^+$) and 627 ($[604 + Na]^+$) exhibited characteristic losses of 112 amu to give ions at *m/z* 489 and 515 (Figure 2C, I and IV). This confirmed that the compounds having molecular weights of 578 and 604 Da are new acetogenins possessing a C-4 hydroxyl group. As listed in Table 2, an acetogenin having both a molecular weight of 578 Da and a C-4 hydroxyl group should be a C_{35} bis-THF compound, as illustrated in Figure 9. The relatively long retention time of this ion peak (55.6 min) is consistent with the polarity expected for an acetogenin having only two hydroxyl groups. Further isolation and identification work on these novel compounds is underway in our laboratory.

The results of the current investigation demonstrate that LC/(+)ESI-MS provides a highly sensitive detection method that can assist in: (1) identification of new

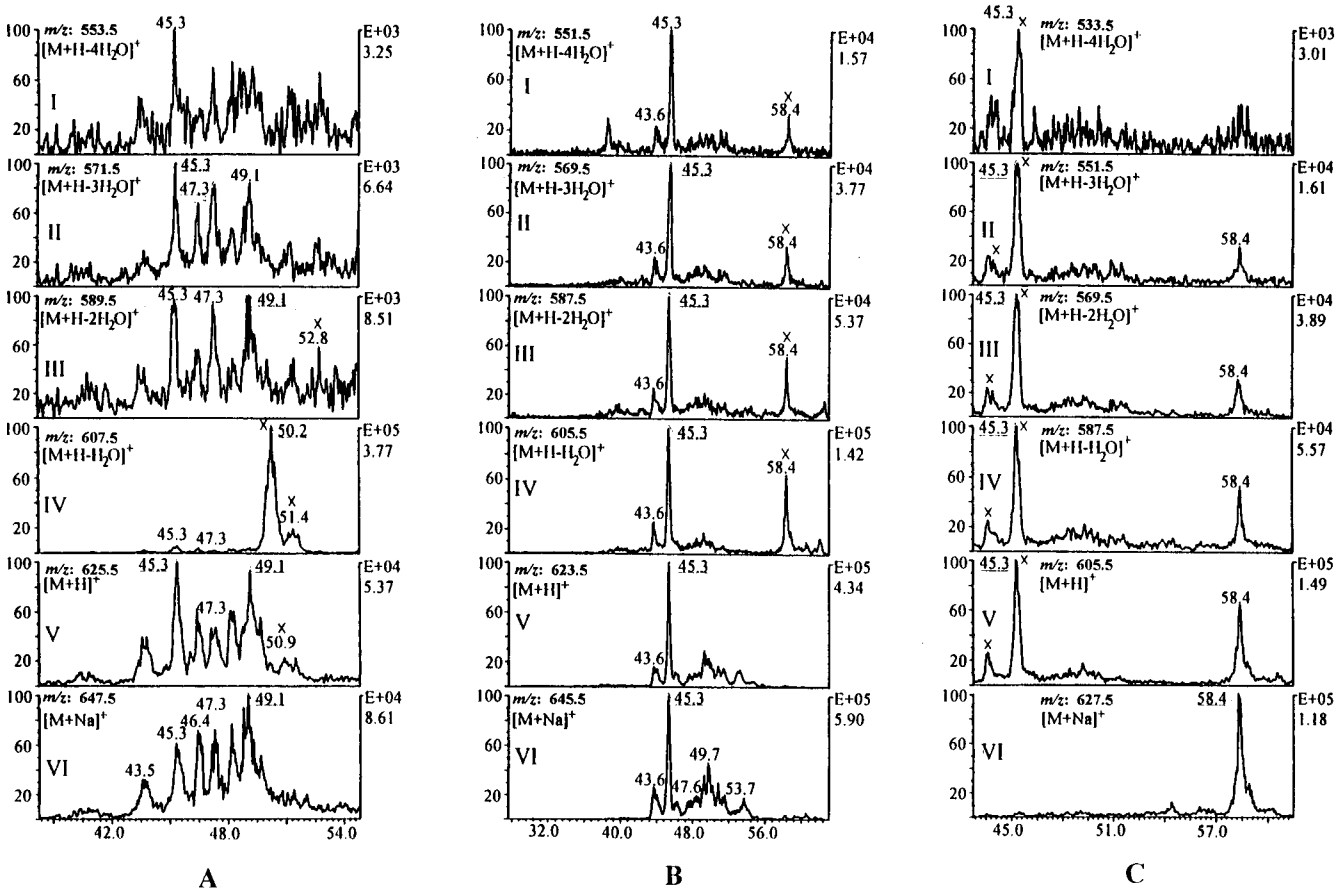


Figure 4. Selected ion chromatograms (SIC) of acetogenins present in a methanol-soluble fraction of *Rollinia mucosa* with molecular weights of 624 (A), 622 (B), and 604 (C) Da. The SIC presented in A−C are as follows: $[M + Na]^+$ (VI), $[M + H]^+$ (V), $[M + H - H_2O]^+$ (IV), $[M + H - 2H_2O]^+$ (III), $[M + H - 3H_2O]^+$ (II), and $[M + H - H_2O]^+$ (I). The peaks marked by "x" were apparently not produced from acetogenins with these molecular weights.

sources of targeted families of natural products; (2) highlighting specific fractions of extracts as meriting greater or less interest based on the novelty of the components; and (3) specific detection of new derivatives in a series of bioactive plant fractions, as shown by the new acetogenins described in this paper.

Experimental Section

General Experimental Procedures. HPLC was performed with a Waters 616 solvent delivery system (Millipore Co., Milford, MA) operated at 0.4 mL/min, a Waters WISP 717 autosampler with gradient flow control, and a 150 × 4.6 mm Zorbax XDB-C_8 column (MAC-MOD Analytical, Chadds Ford, PA). The HPLC mobile phase was methanol (A) and 0.01 M NH_4OAc buffer (pH 4, B) or methanol (A) and 1% formic acid (B), and the samples were eluted using a linear gradient of 50−80% A over 10 min, followed by 80−95% A over 45 min. MS was performed on a Finnigan-MAT TSQ 7000 triple quadrupole mass spectrometer equipped with an electrospray ion (ESI) source (Finnigan MAT, San Jose, CA). The positive-ion mode was employed, and the spray voltage was set at 4.5 kV. The capillary temperature was maintained at 230 °C. The HPLC fluid was nebulized using N_2 as both a sheath gas, at a pressure of 80 PSI, and an auxiliary gas at a flow rate of 30 mL/min.

Materials. The crude methanol-soluble fraction of stem bark of *R. mucosa* (Jacq.) Baill. (Annonaceae) and nine pure acetogenins from the same plant were extracted and/or isolated at Purdue University.[9−12] The methanol-soluble fraction was dissolved in methanol at a concentration of 0.1 μg/μL, and 20 μL was injected for each LC/MS analysis. The pure acetogenins were reconstituted to 1.0 ng/μL or 1.0 pg/μL, and volumes of 2 or 20 μL of the samples were loaded to HPLC for each run.

The general method employed injection of 2 μg of crude methanol-soluble fraction for each LC/MS analysis. The detection limits of LC/(+)ESI-MS were determined to be *ca.* 20 pg. This means that any acetogenin having a concentration over 1/100 000 (20 pg/2 μg) in the methanol fraction could be detected. If 200 g of crude extract is used, any acetogenins present at 2 mg or more can be monitored and the fractionation guided by this LC/MS method for isolation and purification. Usually, 2 mg of a pure natural product is required for structural elucidation and bioassay.

LC/(+)ESI-MS. Positive-ion electrospray ionization MS [(+)ESI-MS] was applied to nine pure acetogenins isolated from *R. mucosa*: bullatalicin, 12-hydroxybullatacin A, 12-hydroxybullatacin B, javoricin, muricatetrocin C, muricatetrocin B, rollinecin A, rollitacin, and sylvaticin (Table 1). A mass range of 400−700 amu was scanned at a rate of 400 amu/s throughout the HPLC gradient eluted with two different solvent systems, methanol−1% formic acid, and methanol−0.01 M NH_4-OAc buffer (pH 4).

LC/(+)ESI-APICID-MS. Atmospheric pressure in-source collision-induced dissociation (APICID) was performed at an elevated potential of the octapole filled with nitrogen to a pressure of *ca.* 1 mTorr. A mass

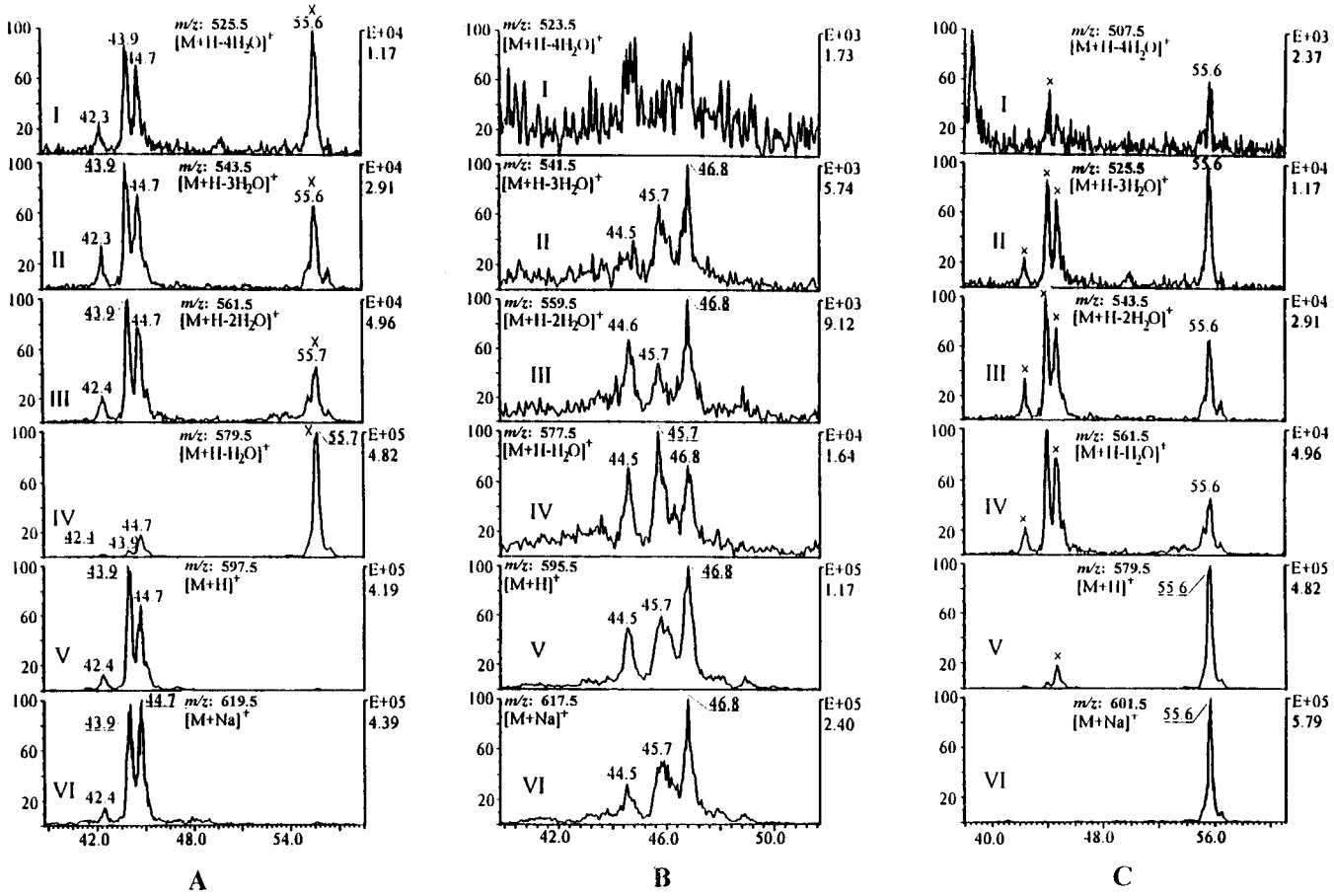
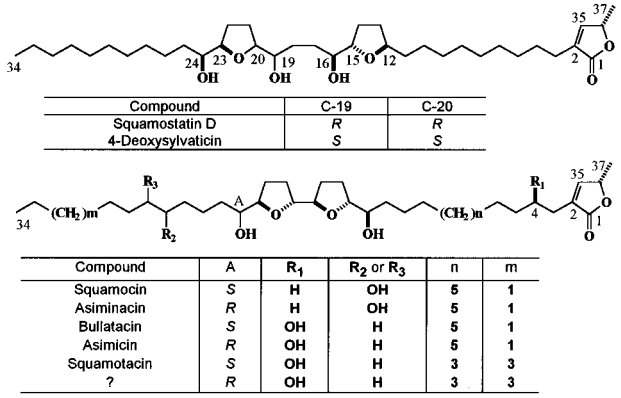


Figure 5. Selected ion chromatograms (SIC) of acetogenins present in a methanol-soluble fraction of *Rollinia mucosa* with molecular weights of 596 (A), 594 (B), and 578 (C) Da. The SIC presented in A−C are as follows: $[M + Na]^+$ (VI), $[M + H]^+$ (V), $[M + H - H_2O]^+$ (IV), $[M + H - 2H_2O]^+$ (III), $[M + H - 3H_2O]^+$ (II), and $[M + H - H_2O]^+$ (I). The peaks marked by "x" were apparently not produced from acetogenins with these molecular weights.

range of 400−700 amu was scanned at a rate of 400 amu/s throughout the HPLC gradient. The potential of the octapole was optimized by carrying out the LC/(+)ESI-APICID-MS measurement of nine pure acetogenins (Table 1) in the range of −5 and −40 V. The best ion patterns were obtained with the octapole potential at −20 V, which was applied to the LC/(+)-ESI-APICID-MS acquisition of all of the pure acetogenins and the methanol-soluble fraction of *R. mucosa*. Methanol and NH_4OAc buffer (0.01 M, pH 4) were used as HPLC mobile phase.

The selected ion chromatogram (SIC) analyses were performed by the software attached to the Finnigan-MAT TSQ 7000 mass spectrometer. After the LC/(+)-ESI-APICID-MS data of the methanol-soluble fraction of *R. mucosa* were obtained, all of the $[M + H]^+$ ions of the possible molecular weights (Table 2) of the acetogenins were searched by inputing each *m/z* value individually. Those that showed peaks in the SICs were further individually analyzed by selecting the ion traces of their respective $[M + Na]^+$, $[M - H_2O + H]^+$, $[M - 2H_2O + H]^+$, $[M - 3H_2O + H]^+$, and $[M - 4H_2O + H]^+$ ions, which was referred to ion pattern comparison.

LC/(+)ESI-MS/MS. The MS/MS measurements were made by selecting the sodium adduct parent ions of acetogenins with a 1 amu-wide window and passing the ions into the collision cell, which was filled with argon at a pressure of 2 mTorr. The fragment ions were scanned at 400 amu/s over a mass range of 50−700 amu. The collision energies were optimized by conducting the daughter ion scans of the nine pure acetogenins (Table



Compound	C-19	C-20
Squamostatin D	*R*	*R*
4-Deoxysylvaticin	*S*	*S*

Compound	A	R_1	R_2 or R_3	n	m
Squamocin	*S*	H	OH	5	1
Asiminacin	*R*	H	OH	5	1
Bullatacin	*S*	OH	H	5	1
Asimicin	*R*	OH	H	5	1
Squamotacin	*S*	OH	H	3	3
?	*R*	OH	H	3	3

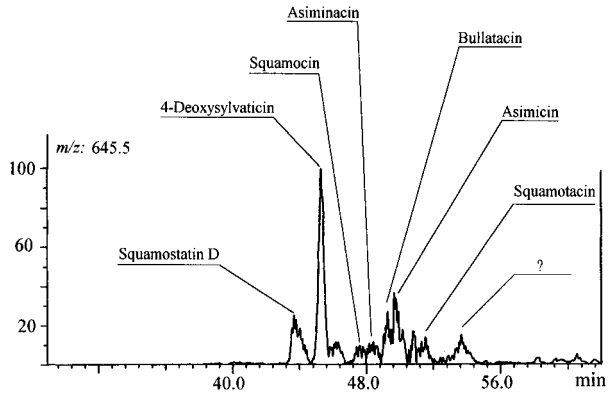


Figure 6. Selected ion chromatogram at *m/z* 645 $[M + Na]^+$ from a methanol-soluble fraction of *Rollinia mucosa* and the possible compounds corresponding to the ion peaks.

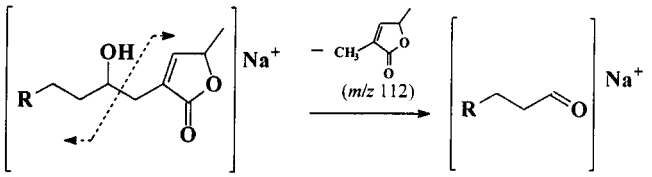


Figure 7. Proposed ion pathway leading to the loss of 112 amu from the acetogenins.

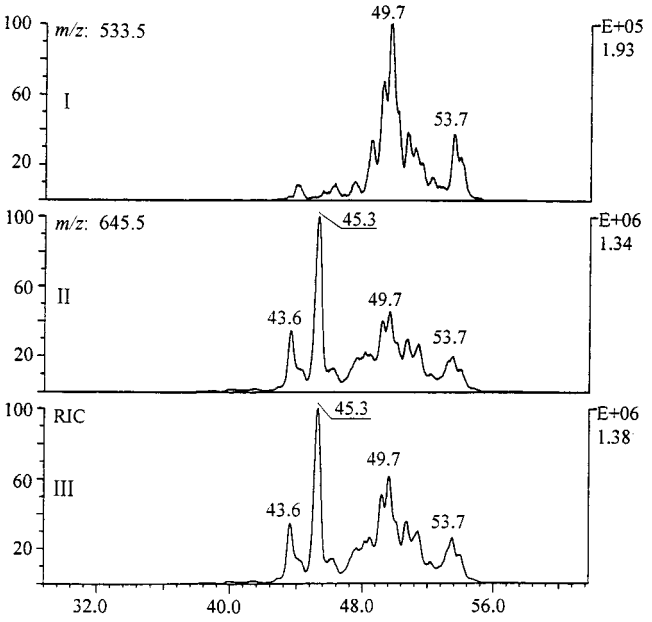


Figure 8. LC/(+)ESI-MS/MS ion chromatograms of the $[M + Na]^+$ daughter ions at *m/z* 645 from a methanol-soluble fraction of *Rollinia mucosa*. (I) Ion chromatogram at *m/z* 533.5 resulting from the loss of 112 from *m/z* 645.5. (II) Selected ion chromatogram at *m/z* 645.5. (III) Reconstructed ion chromatogram (RIC) showing total mass ion between 32 and 56 min.

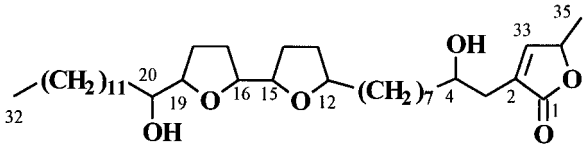


Figure 9. General structure proposed for C-4 hydroxylated acetogenins exhibiting a $[M + H]^+$ ion at *m/z* 579.

1) in a collision energy range of 20−60 eV. The best parent to daughter ion signal ratios were obtained with the collision energy at 45 eV, which was applied to the LC/(+)ESI-MS/MS measurement of all of the pure acetogenins and the methanol-soluble fraction of *R. mucosa*. Methanol and 1% formic acid were used as HPLC mobile phase.

LC/(+)ESI-APICID-MS/MS. The APICID-MS/MS measurements were made by selecting the proton adduct parent ions with a 1 amu-wide window and passing the ions into the collision cell that was filled with argon at a pressure of 2 mTorr. The fragment ions were scanned at 400 amu/s over a mass range of 50−700 amu. The octapole was filled with nitrogen to a pressure of *ca*. 1 mTorr, and the octapole potential was set at −20 V. The collision energies were optimized by conducting the daughter ion scans of the nine pure acetogenins (Table 1) in a collision energy range of 20−60 eV. The best parent-to-daughter ion signal ratios were obtained with the collision energy set at 45 eV, which was applied to the LC/(+)ESI-APICID-MS/MS measurement of all of the pure acetogenins, and the methanol-soluble fraction of *R. mucosa*.

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References and Notes

(1) Lindon, J. C.; Nicholson, J. K.; Wilson, I. D. *Adv. Chromatogr.* **1995**, *63*, 315−382.
(2) Mutlib, A. E.; Strupczewski, J. K.; Chesson, S. M. *Drug Metabol. Dispos.* **1995**, *23*, 951−964.
(3) Wolfender, J.-L.; Hostettmann, K. In *Recent Advances in Phytochemistry*; Arnason, J. T., Mata, R., Romeo, J. T., Eds.; Plenum Press: New York, 1995; Vol. 29, pp 189−216.
(4) Royer, I.; Alvinerie, P.; Armand, J. P.; Ho, L. K.; Wright, M.; Monsarrat, B. *Rapid Commun. Mass Spectrom.* **1995**, *9*, 188−190.
(5) Kerns, E. H.; Volk, K. J.; Hill, S. E.; Lee, M. S. *Rapid Commun. Mass Spectrom.* **1995**, *9*, 1539−1545.
(6) Siuzdak, G. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 11290−11297.
(7) Iwabuchi, H.; Kitazawa, E.; Kobayashi, N.; Watanabe, H.; Kanai, M.; Nakamura, K. *Biol. Mass Spectrom.* **1994**, *23*, 540−546.
(8) Rupprecht, J. K.; Hui, Y.-H.; McLaughlin, J. L. *J. Nat. Prod.* **1990**, *53*, 237−278.
(9) Fang, X.-P.; Reiser, M. J.; Gu, Z.-M.; Zhao, G.-X.; McLaughlin, J. L. *Phytochem. Anal.* **1993**, *4*, 27−67.
(10) Gu, Z.-M.; Zhao, G.-X.; Oberlies, N. H.; Zeng, L.; McLaughlin, J. L. In *Recent Advances in Phytochemistry*; Arnason, J. T., Mata, R., Romeo, J. T., Eds.; Plenum Press: New York, 1995; Vol. 29, pp 249−310.
(11) Zafra-Polo, M. C.; Gonzalez, M. C.; Estornell, E.; Sahpaz, S.; Cortes, D. *Phytochemistry* **1996**, *42*, 253−271.
(12) Shi, G.; Zeng, L.; Gu, Z.-M.; MacDougal, J. M.; McLaughlin, J. L. *Heterocycles* **1995**, *41*, 1785−1796.
(13) Laprévote, O.; Girard, C.; Das, B. C.; Laurens, A.; Cavé, A. *Analysis* **1993**, *21*, 207−210.
(14) Pettit, G. R.; Riesen, R.; Leet, J. E.; Polonsky, J.; Smith, C. R.; Schmidt, J. M.; Dufresne, C.; Schaufelberger, D.; Moretti, C. *Heterocycles* **1989**, *28*, 213−217.
(15) Pettit, G. R.; Cragg, G. M.; Polonsky, J.; Herald, D. L.; Goswami, A.; Smith, C. R.; Moretti, C.; Schmidt, J. M.; Weisleder D. *Can. J. Chem.* **1987**, *65*, 1433−1435.
(16) Shi, G.; Alfonso D.; Fatope, M. O.; Zeng, L.; Gu, Z.-M.; Zhao, G.-X.; He, K.; MacDougal, J. M.; McLaughlin, J. L. *J. Am. Chem. Soc.* **1995**, *117*, 10409−10410.
(17) Shi, G.; Ye, Q.; He, K.; M.; McLaughlin, J. L. *J. Nat. Prod.* **1996**, *59*, 548−551.
(18) Shi, G.; Gu, Z.-M.; He, K.; Wood, K. V.; Zeng, L.; Ye, Q.; MacDougal, J. M.; McLaughlin, J. L. *Bioorg. Med. Chem. Lett.* **1996**, *4*, 1281−1286.
(19) Shi, G.; Kan, H.; Ye, Q.; MacDougal, J. M.; McLaughlin, J. L. *Tetrahedron Lett.*, submitted.

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