Pergamon

Bioorganic & Medicinal Chemistry, Vol. 5, No. 10, pp 1911–1916, 1997

PII: S0968-0896(97)00129-6
0968-0896/97 $17.00 + 0.00

Isolation of New Bioactive Annonaceous Acetogenins from *Rollinia mucosa* Guided by Liquid Chromatography/Mass Spectrometry

Zhe-ming Gu,[a] Dawei Zhou,[a] Neil J. Lewis,[a] Jinn Wu,[a,*] Guoen Shi[b] and Jerry L. McLaughlin[b]
[a]*XenoBiotic Laboratories, Inc., 107 Morgan Lane, Plainsboro, NJ 08536, U.S.A.*
[b]*Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN 47907, U.S.A.*

Abstract—Reversed-phase high-performance liquid chromatography (RP-HPLC) fractionation, monitored by liquid chromatography/electrospray mass spectrometry (LC/ESI-MS), led to the isolation of two new bioactive annonaceous acetogenins, rollidecin C (**1**) and rollidecin D (**2**), from the bioactive aqueous methanol fraction of the leaves of *Rollinia mucosa* (Annonaceae). The structures were confirmed by analyses of the ^1H and ^{13}C NMR data. In addition, a known adjacent *bis*-tetrahydrofuran (THF) acetogenin, desacetyluvaricin (**3**), was isolated from this plant for the first time utilizing the LC/ESI-MS monitoring approach. Compound **1** exhibited selective cytotoxicity toward the colon tumor cell line (HT-29), while **2** showed only borderline cytotoxicity in a panel of six human tumor cell lines. © 1997 Elsevier Science Ltd.

Introduction

Since 1982, more than 220 annonaceous acetogenins have been reported.[1] Recently, novel acetogenins bearing new structural features [e.g., tetrahydropyran (THP) rings and hydroxyl groups located on the tetrahydrofuran (THF) rings] have been isolated from *Rollinia mucosa* (Jacq.) Baill. (Annonaceae).[2] The diversity of structures and selectivity of the biological actions of the acetogenins, particularly against specific human tumor cell lines, have stimulated a continuing search for new members of this class of potent botanical products.

The annonaceous acetogenins do not exhibit intense UV absorbance, which makes it difficult to locate them in chromatographic fractions of plant extracts. In a previous paper,[3] we demonstrated that the acetogenins are highly sensitive to the positive ion mode of electrospray liquid chromatography-mass spectrometry [LC/(+)ESI-MS]. Utilizing the atmospheric pressure in-source collision induced dissociation ([LC/(+)ESI-APICID-MS]), acetogenins produce characteristic ion patterns consisting of $[M + Na]^+$, $[M + H]^+$, as well as ions showing consecutive losses of H_2O from $[M + H]^+$. In a total ion scan using this method ([LC/(+)ESI-APICID-MS]), an injection of 2 μg of the bioactive aqueous methanol fraction of *R. mucosa* produced more than 40 ion peaks corresponding to acetogenins. The resultant molecular weights of the acetogenin components, combined with HPLC retention times as an indication of polarity, provide a basis for deducing structural characteristics. Thus, LC/MS monitoring offers an efficient and effective method of isolation and purification of acetogenins.

As an example of the utility of this method, we describe the LC/(+)ESI-MS directed isolation of two new bioactive acetogenins, rollidecin C (**1**) and rollidecin D (**2**), in conjunction with the known acetogenin, desacetyluvaricin (**3**), from an aqueous methanol fraction of *R. mucosa* leaves. All structures were confirmed by 1-D and 2-D NMR experiments.

Results and Discussion

When screening for acetogenins from bioactive leaf extracts of *R. mucosa* by LC/(+)ESI-APICID-MS, selected ion chromatograms (SIC) at retention time (t_R) 55.6 min were consistent with the known ion pattern of annonaceous acetogenins.[3] The M_r of the compound with t_R at 55.6 min, designated as **1**, was determined to be 578 based on respective proton ($[M + H]^+$) and sodium ($[M + Na]^+$) adduct molecular ions at *m/z* 579 and 601. In the LC/(+)ESI-MS/MS experiment, the ion of **1** at *m/z* 601 ($[M + Na]^+$) exhibited a loss of 112 amu, showing a fragmentation typical of acetogenins with a 4-hydroxyl group (OH-4).[4] This observation indicated that **1** was an acetogenin. Acetogenins with a M_r of 578 can be either a C_{35} *bis*-THF compound having two hydroxyl groups, or a C_{35} mono-THF compounds having three hydroxyl groups and a double bond.[3] Compound **1** showed a retention time approximately 7 min longer than bullatacin (t_R ca. 49 min, M_r 622), which is a *bis*-THF acetogenin with three hydroxyl groups.[1] The lower polarity relative to bullatacin suggested that **1** had only two hydroxyl groups. C_{35} *bis*-THF acetogenins with a M_r of 578 have only two possible structure types as illustrated in Figures 2(A) and (B), respectively. The key structural difference between these two types is either the presence or absence of an OH-4. Since an OH-4 was

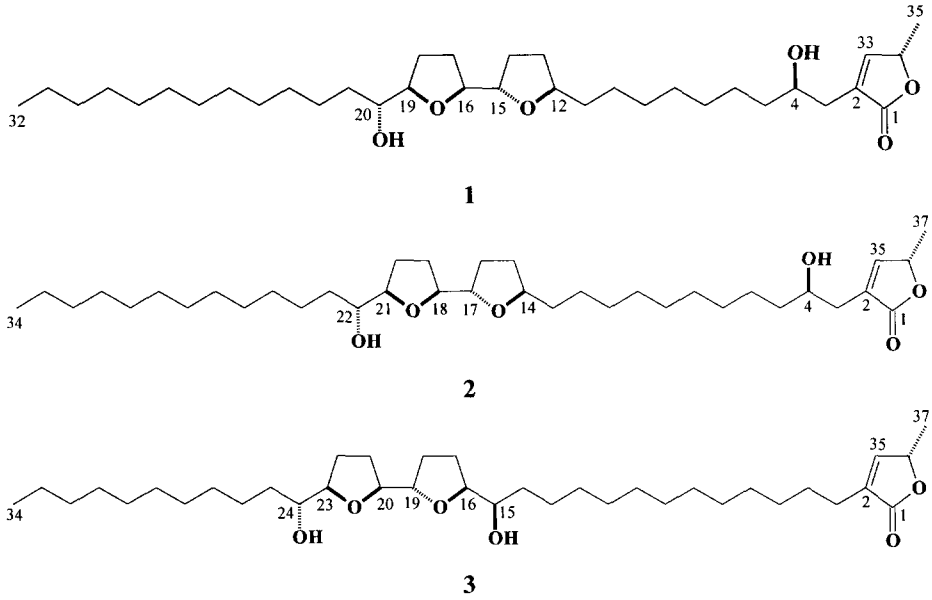

Figure 1. Structures of rollidecin C (**1**), rollidecin D (**2**), and desacetyluvaricin (**3**).

detected in **1** by LC/(+)ESI-MS/MS, the structure shown in Figure 2(A) is proposed for **1**.

The LC/(+)ESI-APICID-MS data for the t_R 58–61 min fractions showed a broad total ion peak with selected ions $[M + Na]^+$ and $[M + H]^+$ at *m/z* 629 and 607, respectively, which yielded a M_r of 606. The daughter ion scan of the $[M + Na]^+$ at *m/z* 629 produced an unexpected result. While the second half of this ion peak produced an intense daughter ion at *m/z* 517, with a typical loss of 112 amu, the first half did not. This result suggested the presence of two different types of acetogenins in these fractions and that they could not be differentiated by UV detection. Compounds **2** and **3** were assigned to the components in the second and first halves of this peak, respectively. The appearance of a molecular ion 28 amu higher than **1** suggested that **2** and **3** had two additional CH_2 units and should be C_{37} acetogenins. Under RP-HPLC conditions, **2** and **3** showed slightly less polarity (t_R 24 min longer) than **1**, supporting the proposed structures with a longer hydrocarbon chain. Similar chromatographic behaviors for **2** and **3**, as compared to **1**, suggested that they also had two hydroxyl groups. Dihydroxylated C_{37} *bis*-THF acetogenins with a M_r of 606 have two different structural types. One has an OH-4 and a hydroxyl group flanking one side of the adjacent *bis*-THF rings (Fig. 2C), whereas, the other type does not have an OH-4, but contains two hydroxyl groups with one flanking each side of the adjacent *bis*-THF rings (Fig. 2D). Since an OH-4 group was detected in **2** by LC/(+)ESI-MS/MS, **2** was proposed to have a structure analogous to that shown in Figure 2(C). Consequently, the structure shown in Figure 2(D) was suggested for **3**, based on the absence of an OH-4. The proposed structures of **2** and **3** were further supported by their polarities, with the observation that shifting a hydroxyl group from C-4 to other positions along the hydrocarbon chain increases polarity.[1,3]

We therefore deduced that both **1** and **2** belong to the same type of acetogenin, which has adjacent *bis*-THF rings with only one flanking hydroxyl group. This type of acetogenin is relatively rare, and to date only a few have been reported.[1d] Two such acetogenins, rollidecins A and B, were recently isolated from the same plant material,[5] but **1** and **2** were known to be different from them based on their HPLC behaviors. If the structures

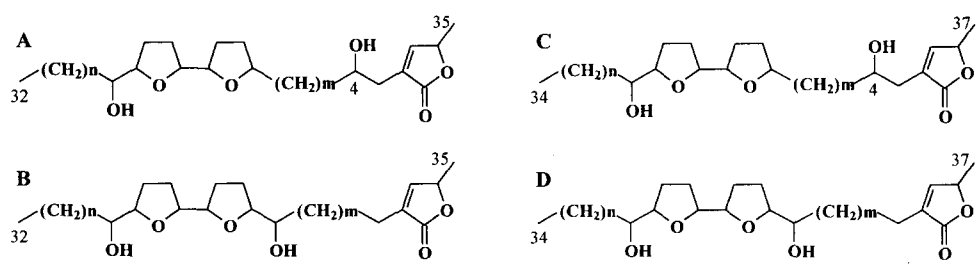

Figure 2. Possible structures of *bis*-THF acetogenins having a M_r of 578 (A and B, $m + n = 18$) or 606 (C and D, $m + n = 20$).

proposed in Figures 2(A) and (C) were correct, **1** and **2** would be new compounds. Thus, fractions containing **1** were combined and chromatographed by RP-HPLC to give a white waxy solid. The mixture of **2** and **3** was further fractionated by RP-HPLC and monitored by LC/MS/MS. Fractions containing mainly **2** or **3** were combined accordingly and further purified by RP-HPLC. After an additional RP-HPLC run, **2** and **3** were completely resolved to yield a white waxy solid (**2**) and a white powder (**3**).

The NMR spectra of **3** were consistent with *threo–trans–threo–trans–erythro* relative stereochemistries around the *bis*-THF rings from C-15 to C-24, and were identical to those of desacetyluvaricin. While desacetyluvaricin has been previously isolated from *Uvaria acuminata*,[6] *Annona bullata*,[7] and *Annona squamosa*,[8] this represents the first isolation from *R. mucosa*.

The ^{1}H and ^{13}C NMR spectra of **1** (Table 1) confirmed the proposed structure. The proton signals at δ 7.19 (q, H-33), 5.06 (qq, H-34), 3.84 (m, H-4), 2.53 (m, H-3a), 2.40 (m, H-3b), and 1.43 (d, H-35), and the carbon resonances at δ 174.5 (C-1), 151.7 (C-33), 131.2 (C-2), 78.0 (C-34), 69.9 (C-4), and 19.1 (C-35) are characteristic for an α,β-unsaturated γ-lactone moiety with an OH-4 group.[1] The presence of the adjacent bis-THF rings was indicated by four proton signals at δ 3.92–3.98 and four carbon resonances at δ 80.1–82.5 (C-12, 15, 16, and 19).[1] There was only one proton signal at δ 3.45 corresponding to a methine proton attached to a hydroxylated carbon (C-20). In the ^{1}H–^{1}H COSY spectrum of **1**, this proton showed cross peaks with one of the oxygenated THF ring methine protons at δ 3.92–3.98. The results suggested a THF-flanking hydroxyl group at C-20. The absence of a flanking hydroxyl group on the other side of the adjacent bis-THF rings was indicated by the characteristic carbon signals at δ 35.8 (C-11), 80.1 (C-12), and 32.2 (C-13)[1,9] The location of the adjacent bis-THF ring system at C-12 to C-19 was determined by the EIMS fragmentation (Fig. 3).

The proton (δ 3.45) and carbon (δ 74.7) chemical shifts of the THF flanking carbinol center (C-20) of **1** suggested a *threo* configuration between this secondary hydroxyl-bearing carbon and the THF ring (C-19/20), by comparisons of the NMR data with those of model compounds and known acetogenins.[1,10] The relative stereochemistry (*cis* or *trans*) across THF rings can be determined by several different methodologies.[1] However, the best way is to compare the chemical shifts of the two pairs of methylene protons in the THF rings. NMR data are available for a pair of previously synthesized adjacent *bis*-THF rings with *trans–threo–trans* and *trans–threo–cis* relative stereochemistries.[11] The chemical shifts of the H_a and H_b of the methylenes in the second THF ring were recorded at δ 1.98 and 1.65 for a *trans*-THF ring, and at δ 1.82 and 1.94 for the corresponding *cis*-THF ring. Fujimoto *et al.* reported similar NMR data resulting from a series of 2,5-sub-

Table 1. NMR data for **1** and **2** (500 MHz for ^{1}H and 75 MHz for ^{13}C, in $CDCl_3$)

	Rollidecin C (1)		Rollidecin D (2)	
Position	**^{1}H**	**^{13}C**	**^{1}H**	**^{13}C**
1		174.5		174.6
2		131.2		131.2
3	2.40m, 2.53m	33.3	2.40m, 2.53m	33.4
4	3.84m	69.9	3.84m	70.1
5	1.20–1.50m	37.4	1.20–1.50m	37.5
6	1.20–1.50m	25.4	1.20–1.50m	25.6
7–9 (**1**); 7–11 (**2**)	1.20–1.50m	29.1–29.7	1.20–1.50m	29.1–29.8
10 (**1**); 12 (**2**)	1.20–1.50m	26.1	1.20–1.50m	26.2
11 (**1**); 13 (**2**)	1.20–1.50m	35.8	1.20–1.50m	35.9
12 (**1**); 14 (**2**)	3.92–3.98m	80.1	3.92–3.98m	80.2
13 (**1**); 15 (**2**)	2.04m, 1.50m	32.2	2.04m, 1.50m	32.2
14 (**1**); 16 (**2**)	2.04m, 1.50m	28.6[a]	2.04m, 1.50m	28.8[a]
15 (**1**); 17 (**2**)	3.92–3.98m	80.7	3.92–3.98m	80.7
16 (**1**); 18 (**2**)	3.92–3.98m	81.5	3.92–3.98m	81.6
17, 18 (**1**); 19, 20 (**2**)	1.80–1.95m	28.5,[a] 28.4[a]	1.80–1.95m	28.5[a]
19 (**1**); 21 (**2**)	3.92–3.98m	82.5	3.92–3.98m	82.6
20 (**1**); 22 (**2**)	3.36m	74.7	3.36m	74.8
21 (**1**); 23 (**2**)	1.20–1.50m	34.5	1.20–1.50m	34.6
22 (**1**); 24 (**2**)	1.20–1.50m	25.8	1.20–1.50m	25.9
23–29 (**1**); 25–31 (**2**)	1.20–1.50m	29.1–29.7	1.20–1.50m	29.1–29.8
30 (**1**); 32 (**2**)	1.20–1.50m	31.9	1.20–1.50m	31.9
31 (**1**); 33 (**2**)	1.20–1.50m	22.6	1.20–1.50m	22.7
32 (**1**); 34 (**2**)	0.88t (J = 7.0)	14.0	0.88t (J = 7.0)	14.1
33 (**1**); 35 (**2**)	7.19m	151.7	7.19m	151.8
34 (**1**); 36 (**2**)	5.06m	78.0	5.06m	78.0
35 (**1**); 37 (**2**)	1.43d (J = 6.9)	19.1	1.43d (J = 6.9)	19.1

[a]Indicates interchangeable assignments within the columns.

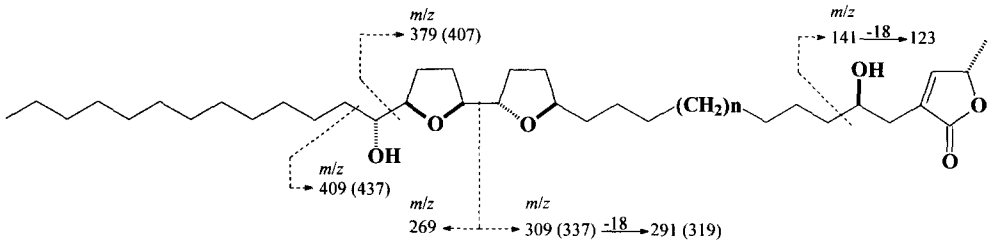

Figure 3. The EIMS fragmentation of 1 (n = 1) and 2 (n = 3).

stituted mono-THF rings with known configurations.[12] In a five-membered ring, larger functional groups are energetically more favorable in an equatorial position. Thus, a *trans*-2,5-substituted THF ring will take an envelope conformation and the methylene protons on the equatorial bonds would be expected to shift to lower field than those on the axial bonds. By comparison, a *cis*-2,5-substituted THF ring must assume a conformation close to planar to keep both substituents in equatorial positions. Thus, the two methylene protons would be positioned in very similar magnetic fields. The well separated signals, at δ 2.04 and 1.50, of the H_a and H_b of C-13 and C-14 in the first THF ring (C-12 to C-15) indicated a *trans* relative stereochemistry across C-12/15. A *cis* configuration was assigned to the second THF ring (C-16 to C-19) based on the chemical shifts of H_a and H_b of C-17 and C-18 at δ 1.80–1.95.

The absolute configurations of the hydroxylated carbon centers of acetogenins can be readily determined by Mosher's ester methodology,[13] allowing the absolute configurations of other stereogenic centers in the THF moieties to be assigned by analysis of the relative stereochemistries.[1,14] The preparation of Mosher's esters has defined the absolute configuration of over half of the currently known acetogenins.[1c,1d] With the exception of the C-2 position of the ketolactone acetogenins, which are thought to arise as artifacts, the acetogenins are optically pure with no enantiomeric isomerism found.[1c] These findings indicate that the epoxide openings in plants are enzymatically effected by a stereospecific pathway. Based on the formation and absolute configuration of the THF moieties, acetogenins have been grouped into types.[1c,1d] Comparison of the spectral data for most new acetogenins with spectra of the known types allows for assignment of both relative and absolute configurations.

The biogenetic origins of some major types of acetogenins reported from *R. mucosa* are proposed in Figure 4. Compound **1** may originate from the same biogenetic pathways as rollidecins A and B, with concomitant formation of the adjacent *bis*-THF moiety. The ^1H and ^{13}C NMR data of the THF moiety of **1** were virtually identical to those of rollidecins A and B, which were isolated from the same plant fraction.[5] Thus, **1** was concluded to be a new member of the rollidecin type and named rollidecin C with the proposed structure as illustrated.

As shown in Table 1, the ^1H and ^{13}C NMR data of **2** are nearly identical to those of **1**, thereby suggesting that **2** is also a new acetogenin of the rollidecin type. The skeleton of **2** was determined by the EIMS (Fig. 3), and

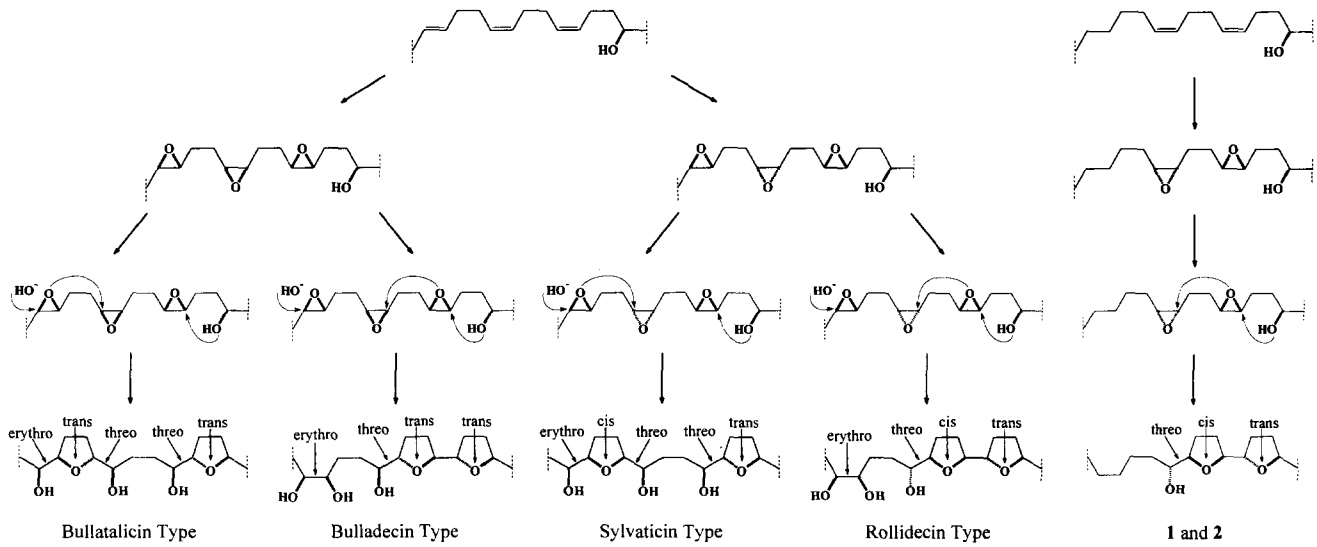

Figure 4. Proposed biogenetic pathways of the THF rings of rollidecins and related types of annoaceous acetogenins.

the structure of **2** was proposed as illustrated. The difference between **2** and **1** is the presence of two additional CH_2 groups between the γ-lactone ring and the adjacent *bis*-THF rings in **2**. Compound **2** was named rollidecin D.

Compounds **1** and **2** showed moderate cytotoxicity against six human tumor cell lines in a seven-day MTT in vitro cytotoxicity panel (Table 2).[15] Compound **1** was uniformly more potent than **2** and showed a selectivity toward the colon cell line (HT-29) with a potency approaching that of adriamycin.

Experimental

Instruments

Melting points were determined on a Fisher–Johns apparatus and were not corrected. The IR and UV spectra were obtained using Perkin–Elmer 1600 FTIR and Beckman DU-7 UV spectrophotometers, respectively. The NMR experiments were carried out on a Varian-500S (^1H at 500 MHz, ^{13}C at 125 MHz) or a Bruker-300 spectrometer (^1H at 300 MHz, ^{13}C at 75 MHz) using $CDCl_3$ as solvent and TMS as reference. HRFABMS was taken on a Kratos MS 50 spectrometer. EIMS was conducted on a VG Trio-1 quadrupole mass spectrometer.

LC/(+)ESI-MS: 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 mm × 4.6 mm Zorbax XDB-C_8 column (MAC-MOD Analytical, Chadds Ford, PA). The mobile phase was NH_4OAc buffer (0.01 M, pH 4, A) and methanol (B), and the sample was eluted using a linear gradient of 50% to 80% B, in 10 min followed by 80% to 95% B, in 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, spray voltage set at 4.5 eV and capillary temperature 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. LC/(+)ESI-APICID-MS: Atmospheric pressure in-source collision induced dissociation (APICID) was performed under the following conditions: potential of the octapole at −20 V, nitrogen pressure at ca. 1 mtorr, scan rate at 400 amu/s, and an ion range of 400–700. LC/(+)ESI-MS/MS: tandem MS measurements were made by selecting the parent ion 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 energy was set at 45 eV.

Plant material

The leaves of *Rollinia mucosa* (Jacq.) Baill. were collected in the Conservatory of the Missouri Botanical Garden, St Louis, MO; the associated plant identification numbers are: MBG 891568, voucher *Sherman* 285 (MO).

Extraction and purification procedures

Preparation of the methanol fraction (F005) of the leaves of *R. mucosa* and the first open column chromatography of this fraction were described in detail in a previous paper.[9] Fractions 3 and 4 from the first open column were combined and loaded to a C_8 HPLC column eluted with methanol and water. Eluate was collected by a fraction collector at an interval of 0.5 min. Aliquots of each fraction were subjected to LC/MS by loop injection. The fractions (t_R ca. 56 min) showing ions *m/z* 579 and 601 were pooled and further purified by another RP-HPLC to give **1**. The fractions at t_R 58–61 min produced $[M + H]^+$ and $[M + Na]^+$ at *m/z* 607 and 629, respectively. In LC/(+)ESI-MS/MS experiments of ion *m/z* 629 ($[M + Na]^+$), the first three fractions did not produce a significant daughter ion at *m/z* 517, whereas, the last three fractions yielded an intense daughter ion at *m/z* 517. Fractions were pooled separately based on the presence or absence of the 112 amu loss and further chromatographed twice by RP-HPLC eluting with acetonitrile and water and monitoring by LC/(+)ESI-MS/MS. For each elution, fractions

Table 2. Cytotoxicities[a] of **1** and **2**[15]

	A-549[b] ED_{50}	MCF-7[c] ED_{50}	HT-29[d] ED_{50}	A-498[e] ED_{50}	PC-3[f] ED_{50}	PACA-2[g] ED_{50}
1	1.32	1.07	6.26×10^{-2}	1.44	2.86×10^{-1}	1.08×10^{-1}
2	5.87	5.05	5.39	3.99	1.90	1.03
Adriamycin[h]	7.12×10^{-3}	1.63×10^{-1}	2.81×10^{-2}	2.50×10^{-3}	3.66×10^{-2}	2.19×10^{-3}

[a]Results are reported in μg/mL; all samples were tested in the same run in each cytotoxicity bioassay (b–g).
[b]Human lung carcinoma.
[c]Human breast carcinoma.
[d]Human colon adenocarcinoma.
[e]Human renal carcinoma.
[f]Human prostatic adenocarcinoma.
[g]Human pancreatic carcinoma.
[h]Adriamycin was used as the standard positive control.

producing $[M + Na]^+$ at *m/z* 629 showing no loss of 112 amu were combined (**A**), as were fractions producing fragmentation of a loss of 112 amu (**B**). Fractions **A** and **B** were dried separately to yield **3** and **2**, respectively.

Bioassays

Seven-day MTT in vitro cytotoxicity tests against six human solid tumor cell lines were carried out at the Purdue Cancer Center, using standard protocols[14] for A-549, MCF-7, HT-29, A-498, PC-3, and PACA-2 with adriamycin as the positive control.

Rollidecin C (**1**) was obtained as a white waxy solid (5 mg); mp 42–43 °C; IR (dry film): 3345, 2936, 2859, 1741, 1674, 1071 cm^{-1}; UV (MeOH) λ_{max} = 222 nm (log ε = 3.50); HRFABMS MH^+ ion found *m/z* 579.4612 (calcd 579.4625), corresponding to $C_{35}H_{62}O_6$; EIMS data (Fig. 3, percentage intensities are indicated in parentheses): 409 (5), 379 (8), 309 (100), 291 (4), 141 (2), 123 (5); NMR and bioactivity data are presented in Tables 1 and 2, respectively.

Rollidecin D (**2**) was obtained as a white waxy solid (5 mg); mp 41–42 °C; IR (dry film): 3345, 2936, 2859, 1741, 1674, 1071 cm^{-1}; UV (MeOH) λ_{max} = 222 nm (log ε = 3.50); HRFABMS MH^+ ion found *m/z* 607.4919 (calcd 607.4938), corresponding to $C_{37}H_{66}O_6$; EIMS data (Fig. 3, percentage intensities are indicated in parentheses): 437 (4), 407 (4), 337 (60), 319 (7), 141 (10), 123 (30); NMR and bioactivity data are presented in Tables 1 and 2, respectively.

Acknowledgements

This work was supported in part by R01 grant no. CA30909 from the National Cancer Institute, National Institutes of Health.

References

1. (a) Rupprecht, J. K.; Hui, Y.-H.; McLaughlin, J. L. *J. Nat. Prod.* **1990**, *53*, 237. (b) Fang, X.-P.; Reiser, M. J.; Gu, Z.-M.; Zhao, G.-X.; McLaughlin, J. L. *Phytochem. Anal.* **1993**, *4*, 27. (c) 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: New York, **1995**, Vol. 29, p 249. (d) Zeng, L.; Ye, Q.; Oberlies, N. H.; Shi, G.; Gu, Z.-M.; He, K.; McLaughlin, J. L. *Nat. Prod. Reports* **1996**, *13*, 275.

2. (a) 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. (b) Shi, G.; Kozlowski, J. F.; Schwedler, J. F.; Wood, K. V.; MacDougal, J. M.; McLaughlin, J. L. *J. Org. Chem.* **1996**, *61*, 7988.

3. Gu, Z.-M.; Zhou, D.; Wu, J.; Shi, G.; Zeng, L.; McLaughlin, J. L. *J. Nat. Prod.* **1997**, *60*, 242.

4. Laprvote, O.; Girard, C.; Das, B. C.; Laurens, A.; Cav, A. *Analysis* **1993**, *21*, 207.

5. Shi, G.; Gu, Z.-M.; He, K.; Wood, K. V.; Zeng, L.; Ye, Q.; MacDougal, J. M.; McLaughlin, J. L. *Bioorg. Med. Chem.* **1996**, *4*, 1281.

6. Jolad, S. D.; Hoffmann, J. J.; Cole, J. R. *J. Nat. Prod.* **1985**, *48*, 644.

7. Gu, Z.-M.; Fang, X.-P.; Rieser, M. J.; Hui, Y.-H.; Miesbauer, L. R.; Smith, D, L.; Wood, K. V.; McLaughlin, J. L. *Tetrahedron* **1993**, *49*, 747.

8. Sahai, M.; Singh, S.; Singh, M.; Gupta, Y. K.; Akashi, S.; Yuji, R.; Hirayama, K.; Asaki, H.; Araya, H.; Hara, N.; Eguchi, T.; Kakinuma, K.; Fujimoto, Y. *Chem. Pharm. Bull.* **1994**, *42*, 1163.

9. Gu, Z.-M.; Fang, X.-P.; Zeng, L.; Kozlowski, J. F.; McLaughlin, J. L. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 473.

10. Born, L.; Lieb, F. J.; Lorentzen, P.; Moeschler, H.; Nonfon, M.; Sllner, R.; Wendisch, D. *Planta Med.* **1990**, *56*, 312.

11. Gu, Z.-M.; Fang, X.-P.; Zeng, L.; Song, R.; Ng, J. H.; Wood, K. V.; Smith, D. L.; McLaughlin, J. L. *J. Org. Chem.* **1994**, *59*, 3472.

12. Fujimoto, Y.; Murasaki., C.; Shimada, H.; Nishioka, S.; Kakinuma, K.; Singh, S.; Singh, M.; Gupta, Y. K.; Sahai, M. *Chem. Pharm. Bull.* **1994**, *42*, 1175.

13. (a) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512–519. (b) Sullivan, G. R.; Dale, J. A.; Mosher, H. S. *J. Org. Chem.* **1973**, *38*, 2143. (c) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092.

14. Rieser, M. J.; Hui, Y.-H.; Rupprecht, J. K.; Kozlowski, J. F.; Wood, K. V.; McLaughlin, J. L.; Hanson, P. R.; Zhuang, A.; Hoye, T. R. *J. Am. Chem. Soc.* **1992**, *114*, 10203.

15. The seven-day MTT in vitro cytotoxicity tests against human tumor cell lines followed the standard protocols; see: Shi, G.; Zeng, L.; Gu, Z.-M.; MacDougal, J. M.; McLaughlin, J. L. *Heterocycles* **1995**, *41*, 1785.

(*Received in U.S.A.* 3 *March* 1997; *accepted* 2 *June* 1997)