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ROLLINICIN AND ISOROLLINICIN, CYTOTOXIC ACETOGENINS FROM *ROLLINIA PAPILIONELLA*

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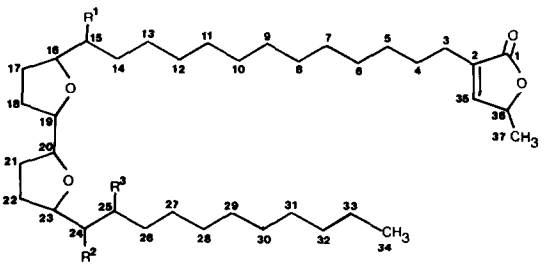
Key Word Index—*Rollinia papilionella*; Annonaceae; linear acetogenins; rollinicin; isorollinicin.

Abstract—Rollinicin and isorollinicin, two new linear acetogenins bearing bistetrahydrofuran moieties and an α,β-unsaturated-γ-lactone, were isolated from roots of *Rollinia papilionella*. The structure elucidation of rollinicin and isorollinicin was achieved through interpretation of the ^{1}H NMR, ^{13}C NMR and high resolution mass spectra.

INTRODUCTION

As part of a program to isolate potential antineoplastic agents from plants, a 95% ethanol extract of *Rollinia papilionella* was found to exhibit significant activity *in vivo* and *in vitro* against the P388 lymphocytic leukemia [1]. During the course of fractionation of this extract guided by bioassay against the P388 cell culture, two novel cytotoxic principles, rollinicin (**1**) and isorollinicin (**2**), were isolated and characterized as linear acetogenins bearing a bistetrahydrofuran moiety similar to polyether antibiotics such as septamycin [2], antibiotic A-204A [3] and antibiotic A28605B [4]. To our knowledge, this is the first report of the isolation of this class of compounds from a *Rollinia* species. However, the isolation of a related compound, uvaricin (**3**), from *Uvaria accuminata* (Annonaceae) has recently been reported [5].



	R^1	R^2	R^3
1	OH	OH	OH
2	OH	OH	OH
3	OH	$OCOCH_3$	H
4	$OCOCH_3$	$OCOCH_3$	$OCOCH_3$
5	OTMS	OTMS	OTMS

RESULTS AND DISCUSSION

A 95% ethanol extract of the roots of *R. papilionella* demonstrated significant inhibitory activity *in vivo* against the P388 lymphocytic leukemia in the mouse (3PS) and *in vitro* against cells derived from a human carcinoma of the nasopharynx (9KB) and cells derived from the P388 leukemia (9PS) [1]. Activity guided fractionation (9PS) of the extract showed the activity to be concentrated successively in the chloroform phase of a chloroform–water partition, the 90% methanol phase of a 90% methanol–petrol partition, and the carbon tetrachloride phase of a carbon tetrachloride–80% methanol partition. Extensive column chromatography and preparative TLC led to the isolation of two new cytotoxic principles, rollinicin (**1**) and isorollinicin (**2**).

Rollinicin (**1**) was obtained as a waxy colorless solid with an mp of 30–32°. The high resolution CI mass spectrum of **1** gave an $[M+H]^+$ ion at *m/z* 623.4981, consistent with a molecular formula of $C_{37}H_{66}O_7$. Microanalyses (C and H) supported this molecular formula. Rollinicin was unaffected by aqueous acid, gave a positive Legal test [6], and gave a positive response with Kedde's reagent [7]. These results suggested the presence of an α,β-unsaturated-γ-lactone moiety. The IR spectrum of **1** indicated an α,β-unsaturated lactone with a band at 1775 cm^{-1}, and the UV spectrum supported this conclusion. The ^{1}H NMR spectrum (Table 1) showed a three-proton doublet (J = 6.8 Hz) at δ1.40, a broad two-proton triplet at δ2.26, a one-proton quartet of quartets (J = 1.5, 6.8 Hz) at δ4.99, and a one-proton quartet at δ6.98 (J = 1.5 Hz). These signals are typically found in the ^{1}H NMR spectra of α,β-unsaturated-γ-lactones related to β-angelica lactone, particularly those with long alkyl chains at the carbon to the carbonyl, such as ancepsenolide [8–10]. The ^{13}C NMR spectrum (Table 2) further confirmed the nature of the γ-lactone with resonances at 173.9 (*s*, C=O), 148.9 (*d*, C=CH), 134.1 (*s*, C=CH), 77.4 (*d*, C=C–C–O), 25.4 (*t*, CH_2–C=C) and 19.1 ppm (*q*, CH_3–C–O).

The ^{1}H NMR spectrum of **1** showed a three-proton, unsymmetrical triplet at δ0.88 which, together with a broad signal at δ1.25, suggested a terminal methyl group of a long straight chain saturated hydrocarbon [11]. This was further substantiated by the band at 710 cm^{-1} in the IR spectrum of **1** which was assigned to the rocking vibration band of a long series of methylene groups

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Table 1. ^{1}H NMR chemical shifts (δ) and coupling constants (in parentheses; Hz) for **1**, **2** and **4**

Hydrogens	1	2	4
C-3	2.26 *t**	2.26 *t**	2.28 **t*
C-4	1.54 *m*	1.54 *m*	1.4–2.0 *m*
C-5–13	1.26 *br*	1.26 *br*	1.25 *br*
C-14	1.28 *m*	1.36 *m*	1.4–2.0 *m*
C-15	3.40† *m*	3.40† *m*	4.7–5.1 *m*
C-16	3.86–3.93 *m*	3.86–3.93 *m*	4.7–5.1 *m*
C-17, 18	1 85–1.98 *m*	1.81–2.01 *m*	1.4–2.0 *m*
C-19, 20	3.86–3.93 *m*	3.86–3.93 *m*	3.8–4 2 *m*
C-21, 22	1.85–1.98 *m*	1.81–2.01 *m*	1.4–2.0 *m*
C-23, 24	3.86–3.93 *m*	3.86–3.93 *m*	3.8–4 2 *m*
C-25	3 60† *m*	3.82† *m*	4.7–5.1 *m*
C-26	1.54 *q* (7.5)	1.54 *q*	1.4–2.0 *m*
C-27–33	1.26 *br*	1.26 *br*	1.25 *br*
C-34	0.88 *t* (7)	0.88 *t* (7)	0.87 *t* (7)
C-35	6.98 *q* (1.5)	6.98 *q* (1.5)	6.98 *q*
C-36	4.99 *dq* (1.5, 6.8)	4.99 *dq* (1.5, 6.8)	4.7–5 1 *m*
C-37	1 40 *d* (6.8)	1.40 *d* (6.8)	1.40 *d* (6.8)
MeCO–			2.02 *s*
			2.04 *s*
			2.07 *s*

*This resonance was not sufficiently resolved to measure coupling constants, but is coupled to the hydrogens on C-4 and C-35.

†May be interchanged.

$[(CH_2)_n, n > 4]$, and by the mass spectrum which exhibited five ions from m/z 71 to 127 differing by 14 mu. This was indicative of a fragment with the structure $Me(CH_2)_8-$.

The IR spectrum of rollinicin (**1**) suggested the presence of hydroxyl groups with absorptions at 3590 and 3464 cm^{-1}. The high resolution CI mass spectrum of **1** indicated the presence of three hydroxyl groups through three successive losses of 18 mu to give ions at m/z 605, 587 and 569. This was confirmed by the formation of a triacetate (**4**) upon acetylation of **1** with acetic anhydride–pyridine, and the formation of a tristrimethylsilyl (TMSi) ether derivative (**5**). Finally, the ^{13}C NMR spectrum (Table 2) indicated that the hydroxyl moieties must be on secondary carbons since the resonances assigned to the hydroxyl-bearing carbons at 71.6, 71.5 and 74.1 ppm appeared as doublets in the off-resonance decoupled spectrum.

In addition to the four resonances due to oxygenated carbons already described, the ^{13}C NMR spectrum of **1** showed four resonances at 82.0, 82.5, 82.7 and 83.3 ppm which were also due to carbons bearing oxygen. These resonances all appeared as doublets in the off-resonance decoupled spectrum and, due to the chemical shifts, were assigned to two other moieties of the type >CH–O–CH<. Multiple resonances between δ3.85 and 3.93 in the ^{1}H NMR spectrum were indicative of protons α to oxygen atoms, and, in combination with the ^{13}C NMR data, suggested the presence of a tetrahydrofuran moiety [12].

The complete structure of rollinicin (**1**) was established by examination of the high resolution CI mass spectral fragmentation pattern. The major fragmentation of a compound such as **1** should occur between adjacent oxygenated carbons. In fact, major ions were found at m/z 295.2273 (100%) ($C_{18}H_{31}O_3$) due to cleavage between C-15 and C-16, m/z 169.1578 (15.6%) ($C_{11}H_{23}O_2 - H_2O$) due to cleavage between C-23 and C-24, and m/z 141.0911 (11.8%) ($C_8H_{12}O_2 + H$) due to the bistetrahydrofuran moiety. Fragmentation of the bond between C-19 and C-20 gave major ions at m/z 347.2595 (64.9%) ($C_{22}H_{37}O_4 - H_2O$) and m/z 239.2003 (20.6%) ($C_{15}H_{29}O_3 - H_2O$). These data were almost identical to those reported for uvaricin (**3**) [5], and served to establish the relative positions of the bistetrahydrofuran and two of the three hydroxyl moieties.

The position of the third hydroxyl group was limited to C-25 through C-28 by the presence of an ion at m/z 85.1029 (5.8%) (C_6H_{13}) due to C-29 through C-34, and was presumed to be C-25 due to three additional minor ions at m/z 99 (C_7H_{15}), 113 (C_8H_{17})$_7$ and 127 (C_9H_{19}). These latter ions were too small to measure accurately. The location of the third hydroxyl group on C-25 was confirmed by examination of the fragmentation pattern of the CI mass spectrum of rollinicin triacetate (**4**). Cleavage of the C-19 to C-20 bond in **4** gave ions at m/z 407 (46.3%) and 341 (15.5%). The latter ion was due to the fragment containing C-20 through C-34, and fragmented further in a manner typical of glycol diacetates [13]. Intramolecular rearrangement led to cleavage of the bond between C-24 and C-25 with loss of the tetrahydrofuran moiety [m/z 99 (6%)] and gave an ion at m/z 243 (8.1%) ($C_{14}H_{26}O_3 + H$). This ion would only be possible if the two acetate moieties were on adjacent carbons.

Diagnostic ions due to fragmentation α to carbons bearing TMSi ethers have been used to establish the position of double bonds in fatty acids [14]. Inspection of the CI mass spectrum of the trisTMSi ether of rollinicin

Table 2. ^{13}C NMR chemical shifts (ppm) for **1**, **2** and **4***

Carbon	1	2	4
1	173.9 *s*	173.9 *s*	173.7 *s*
2	134.1 *s*	134.4 *s*	134.3 *s*
3	25.4 *t*	25.1 *t*	25.2 *t*
14	37.5† *t*	33.4† *t*	34.0† *t*
15	71.6‡ *d*	71.5‡ *d*	74.1‡ *d*
16	83.3 *d*	83.2 *d*	81.6 *d*
19	82.7 *d*	82.8 *d*	81.2 *d*
20	82.5 *d*	82.4 *d*	80.4 *d*
23	82.0 *d*	82.1 *d*	80.1 *d*
24	71.5‡ *d*	75.1‡ *d*	75.2‡ *d*
25	74.1‡ *d*	74.1‡ *d*	75.3‡ *d*
26	37.1† *t*	32.5† *t*	33.9† *t*
4–13	24.8 *t*	24.7 *t*	25.5 *t*
17	25.7 *t*	25.6 *t*	27.4 *t*
18	27.4 *t*	27.4 *t*	27.7 *t*
21	28.7 *t*	28.4 *t*	28.1 *t*
22	29.4 *t*	29.3 *t*	29.2 *t*
27–31	29.6 *t*	29.6 *t*	29.3 *t*
			29.5 *t*
			30.9 *t*
32	31.8 *t*	31.9 *t*	31.7 *t*
33	22.5 *t*	22.7 *t*	22.5 *t*
34	14.1 *q*	14.1 *q*	14.0 *q*
35	148.9 *d*	148.7 *d*	148.8 *d*
36	77.4 *d*	77.3 *d*	77.3 *d*
37	19.1 *q*	19.2 *q*	19.2 *q*
$O_2\underline{C}Me$			170.5 *s*
			170.7 *s*
			170.8 *s*
$O_2C\underline{C}H_3$			21.1 *q*

*Chemical shift assignments were made based upon refs. [17] and [18].

†, ‡ May be interchanged within the same column.

(**5**) revealed three prominent ions at *m/z* 367 (40%), 243 (50%) and 229 (16%) which were attributed to cleavage α to C-15 and C-24 to yield fragment ions containing C-1 through C-15, C-16 through C-24, and C-25 through C-34, respectively. These data offered further support for the assignment of the hydroxyl moieties to carbons 15, 24 and 25, and structure **1** for rollinicin.

Isorollinicin (**2**) was obtained as an amorphous, colorless solid with mp 66–68°. The molecular formula of isorollinicin (**2**), established by high resolution CI mass spectrometry and ^{13}C NMR, was identical to that of rollinicin (**1**) ($C_{37}H_{66}O_7$). The same major structural features (α,β-unsaturated-γ-lactone, hydroxyl, olefin, *n*-alkyl chain and methyl groups) were present in **2** as determined by examination of the IR, UV, ^1H NMR and ^{13}C NMR spectra, although slightly different chemical shifts were observed in both NMR spectra (Tables 1 and 2). The high resolution CI mass spectrum of **2** exhibited a fragmentation pattern similar to that of **1** with major ions at the same *m/z* ratios. This correlation of the spectral data of **2** with the data for **1** suggested that the two compounds were most likely stereoisomers.

Examination of the ^{13}C NMR spectrum of **2** showed a change of 4.1 and 4.6 ppm in the resonances of C-14 and C-26 relative to the same resonances in the spectrum of **1**. This was indicative of differences in the stereochemistry at C-15 and C-25, which in turn influence the resonances at the adjacent carbons. Comparison of the CI mass spectrum of **1** and **2** showed marked differences in the relative intensities of the important fragment ions at *m/z* 347 and 169, which contained the C-15 and C-25 hydroxyl groups, respectively, in both compounds. This implied that **1** and **2** are isomers which most likely differ in stereochemistry at C-15 and C-25.

EXPERIMENTAL

General. Mps are uncorr. ^1H NMR spectra were recorded at 89.56 or 360 MHz in $CDCl_3$ using TMS as internal standard. ^{13}C NMR spectra were recorded at 22.5 MHz in $CDCl_3$ using TMS as internal standard. CIMS were obtained at the University of Pennsylvania Mass Spectrometry Center using *i*-C_4H_{10} as reagent gas. TLC and prep. TLC were on silica gel 60 plates (0.25 mm). Roots of *R. papilionella* Diels. (B806512, PR-45518) were collected in Peru in October 1975 and supplied by the Medicinal Plant Resources Laboratory, USDA, Beltsville, MD, in accordance with the program developed by the National Cancer Institute. Biological testing was conducted under the auspices of the National Cancer Institute [1]. Both rollinicin (**1**) and isorollinicin (**2**) exhibited cytotoxicity against the P388 lymphocytic leukemia *in vitro* [1] ($ED_{50} = 2.9 \times 10^{-8}$ and 10^{-2} μg/ml, respectively). Rollinicin triacetate (**4**) was somewhat less cytotoxic than **1** ($ED_{50} = 2.6 \times 10^{-4}$ μg/ml). Both rollinicin (**1**) and isorollinicin (**2**) are currently undergoing testing *in vivo* against the P388 leukemia in mice.

Extraction and isolation. Dried, ground roots (8.8 kg) of *R. papilionella* were extracted with 95% EtOH (Soxhlet) for 24 hr. The resulting extract was concd *in vacuo* to a dark gum which was partitioned as above. A portion (13.1 g) of the CCl_4 soluble material was subjected to CC over silica gel 60 (300 g, EM Labs) and eluted with $CHCl_3$ followed by increasing amounts of MeOH in $CHCl_3$. The fraction which eluted with 5% MeOH in $CHCl_3$ was determined by TLC to contain **1** and several oxoaphorine alkaloids [15]. This fraction was subjected to CC in the same manner two more times, followed by prep. TLC on silica gel developed with Et_2NH–*i*-PrOH–C_6H_5Me (2:5.93) to yield 40 mg **1** as a colorless, waxy solid.

A second portion (6.2 g) of the CCl_4 soluble material was subjected to CC over silica gel and eluted with CH_2Cl_2 containing increasing amounts of EtOAc. The fractions eluted with 50% EtOAc in CH_2Cl_2 were subjected to extensive prep. TLC with Et_2NH–*i*-PrOH–C_6H_5Me (2:5:93) to yield 28 mg **2** as a colorless, waxy solid

Rollinicin (3-[13-[5′-[1,2-*dihydroxyundecyl*]-*octahydro*[2,2′-*bifuran*]-5-*yl*-13-*hydroxytridecyl*]-5-*methyl*-2(5H)-*furanone*) (**1**) Mp 30–32°. $[\alpha]_D^{25}$ +6.8° ($CHCl_3$; *c* 0.2411). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3590, 3464, 2930, 1775, 1463, 1375, 1318, 1090, 710. UV $\lambda_{max}^{CH_2Cl_2}$ nm (log ε): 231 (4.31). CIMS (probe) high resolution *m/z* 623.4981 $[M+H]^+$ calc. for $C_{37}H_{67}O_7$ *m/z* 623.4886. CIMS *m/z* (rel. int.): 623 (4.9), 605 (6 5), 587 (14.2), 569 (15.3), 399 (17.0), 347 (64.9), 329 (14.6), 295 (100), 265 (5.7), 251 (3 6), 239 (20.6), 237 (3 1), 223 (3.2), 209 (3.5), 195 (8.7), 181 (3.9), 169 (15.6), 167 (5.0), 153 (7.1), 141 (11.8), 139 (7.4), 127 (1 2), 125 (10 4), 111 (9 6), 99 (14.9), 97 (29 0), 96 (7.6), 85 (9.9), 71 (6.9). (Found: C, 70 8; H, 10 7. $C_{37}H_{66}O_7$ requires: C, 71.3; H, 10.7%.)

Isorollinicin (3-[13-[5′-[1,2-*dihydroxyundecyl*]-*octahydro*-[2,2′-*bifuran*]-5-*yl*-13-*hydroxytridecyl*]-5-*methyl*-2(5H)-*furanone*) (**2**). Mp 66–68°. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3589, 3460, 2929, 1768, 1468, 1375, 1319, 1074, 716. UV $\lambda_{max}^{CH_2Cl_2}$ nm (log ε): 231 (4.30). CIMS (probe) high resolution *m/z* 623.4752 $[M+H]^+$ calc. for $C_{37}H_{67}O_7$ *m/z* 623.4886. CIMS *m/z* (rel int.): 623 (1 5), 605 (9 8),

587 (9.8), 569 (7.8), 347 (43.3), 329 (9 8), 311 (14.1), 295 (100), 265 (9.5), 251 (7.1), 239 (5.9), 237 (6.2), 223 (4.0), 209 (4.3), 195 (4 6), 181 (5.0), 169 (4.4), 141 (9.5), 111 (12.5), 99 (.6), 85 (10.9), 71 (17 4).

Acetylation of **1**. Acetylation of **1** (58.3 mg) with Ac_2O–pyridine at room temp. for 24 hr gave the triacetate **4** (35.1 mg) as an oil. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2960, 2870, 1770, 1735, 1471, 1380, 1320, 1080, 960, 860, 725. CIMS (probe) *m/z* (rel. int.): 749 $[M+H]^+$ (10.2), 689 (34.3), 629 (11.8), 569 (2.0), 493 (42.4), 433 (26.8), 423 (100), 407 (45.9), 341 (15.2), 329 (5.6), 395 (7.2), 297 (45.9), 295 (17 2), 293 (14.4), 281 (17.7), 257 (22 1), 243 (8.2), 229 (3.8), 209 (6.6), 169 (4.9), 146 (5 2), 129 (17.0), 113 (7 4), 111 (4.7), 99 (6.0).

Trimethylsilylation of **1**. Treatment of **1** (41.5 mg) with HMDS–TMCS–pyridine (6.5:5:4) at room temp. for 24 hr [16] gave tris TMSi ether **5** (29.8 mg) as an oil. CIMS (probe) *m/z* (rel. int.): 912 (0 7), 839 $[M+H]^+$ (7.8), 767 (28.8), 749 (99 6), 677 (59.2), 659 (33.0), 623 (2.5), 605 (7.3), 587 (28 8), 569 (10.3), 509 (6.1), 399 (16), 383 (30), 367 (40), 355 (30), 329 (46), 313 (30), 295 (100), 293 (32), 259 (39), 243 (50), 229 (16), 203 (12), 185 (30), 169 (91), 151 (39), 141 (22), 139 (10), 129 (22).

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