Diepoxyrollin and Diepomuricanin B: Two New Diepoxyacetogenins from *Rollinia membranacea* Seeds

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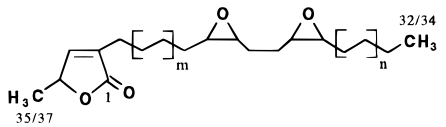
Diepoxyrollin (**1**) and diepomuricanin B (**2**), two new Annonaceous acetogenins were isolated from the cytotoxic MeOH extract of the seeds of Colombian *Rollinia membranacea*. Five others known acetogenins were also isolated, dieporeticanin 1, dieporeticanin 2, diepomuricanin A, rollinone, and sylvaticin. Their structures were elucidated on the basis of spectral data. Compounds **1** and **2** belong to the rare type of diepoxyacetogenins, which are probably precursors of the mono-tetrahydrofuran acetogenins.

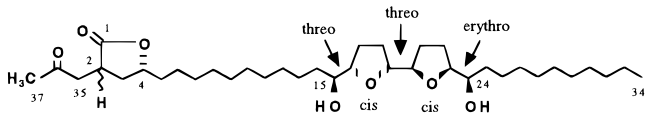
In our previous studies about *Rollinia membranacea* TR. & PL. seeds, we have reported two new adjacent bis-tetrahydrofuran (THF) acetogenins, rioclarin and membranacin,[1] and a new epoxyacetogenin, tripoxyrollin, bearing three epoxy functions in place of THF rings.[2] The epoxyacetogenins, which can be isolated from the less polar fractions of natural extracts of Annonaceae, rank among the natural precursors of mono- or bis-THF acetogenins. Looking for natural antitumor compounds from the seeds of *R. membranacea*, we have succeeded in the isolation and characterization of a series of acetogenins. These include five epoxy-acetogenins having two epoxy functions, diepoxyrollin (**1**), diepomuricanin B (**2**), diepomuricanin A (**3**),[3] dieporeticanins 1 and 2 (**4**,**5**);[4] an "iso"-acetogenin bearing a saturated γ-lactone, rollinone (or iso-rolliniastatin 1) (**6**);[5,6] and a nonadjacent bis-THF acetogenin, sylvaticin (**7**).[7] Two of them, dieporeticanins 1 and 2 (**4**,**5**), were isolated as a mixture; recently, they were also reported as a mixture from *Annona reticulata* seeds, a Vietnamese Annonaceae.[4] In this paper, we describe the isolation and structural elucidation of two new acetogenins, diepoxyrollin (**1**) and diepomuricanin B (**2**).

Diepoxyrollin (**1**) was isolated as a white wax. The molecular weight was indicated by a peak at *m/z* 581 $[M + Li]^+$ and another at *m/z* 575 $[M + H]^+$. HRCIMS (isobutane) from $[M^+]$ *m/z* 574.4900, showed the molecular formula $C_{37}H_{66}O_4$ (calcd 574.4893).

The existence of an unsaturated γ-lactone was first suggested by a positive Kedde reaction, IR carbonyl absorption at 1750 cm^{-1}, and UV λ max at 208 nm. Moreover, the ^{1}H-NMR (200 MHz, $CDCl_3$) signals at δ 6.96 (d, H-35), 4.96 (dq, H-36) and 1.36 (d, H-37) and the ^{13}C-NMR (50 MHz, $CDCl_3$) signals at δ 173.92 (C-1), 134.23 (C-2), 25.10 (C-3), 148.81 (C-35), 77.33 (C-36), and 19.13 (C-37) were typical for an α,β-unsaturated γ-lactone without a hydroxyl group at the C-4 position, a moiety typically found in most acetogenins.[8,9]



1 m= 5; n= 6 ..... $C_{37}H_{66}O_4$
2 m= 6; n= 4 ..... $C_{35}H_{62}O_4$
3 m= 5; n= 5 ..... $C_{35}H_{62}O_4$
4 m= 6, n= 5 ..... $C_{37}H_{66}O_4$
5 m= 7; n= 4 ..... $C_{37}H_{66}O_4$



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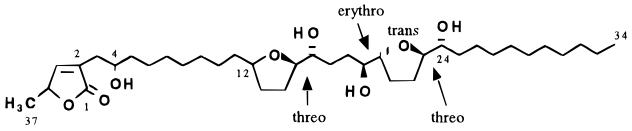

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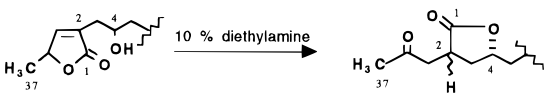

7 **7a**

The existence of epoxides instead of the THF rings in **1** was certified by NMR experiments indicating three multiplets at δ 1.47 (H-14,21), δ 1.65 (H-17,18), and δ 2.94 (H-15,16 and H-19,20). The integration of the multiplet at δ 2.94 for four protons suggested the presence of two epoxide rings. The ^{13}C NMR of **1** showed two resonances due to oxygen-bearing carbons at δ 56.38 and 57.28, integrating for four carbon atoms (C-15,16 and C-19,20) (Figure 1).

The FABMS spectrum with lithium of **1** showed a single peak at *m/z* 581 in the molecular ion region corresponding to the lithiated molecule. The location of the epoxy rings along the alkyl chain of **1** was deduced

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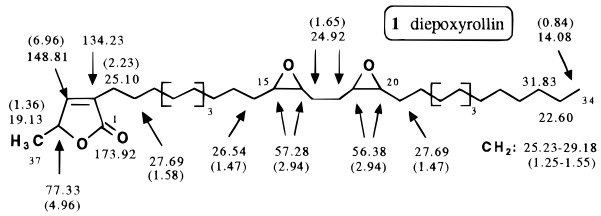

Figure 1. ^{13}C-NMR data* (^{1}H NMR** in parentheses) of diepoxyrollin (**1**).*** *Chemical shifts within a difference $\Delta\delta$ < 1 ppm, may be inverted. **J_{3-4} = 7.5 Hz; J_{3-35} = 1.5 Hz; J_{33-34} = 7.5 Hz; J_{35-36} = 1.5 Hz; J_{36-37} = 7.5 Hz. ***^{1}H and ^{13}C NMR are very similar to those of diepomuricanin B (**2**).

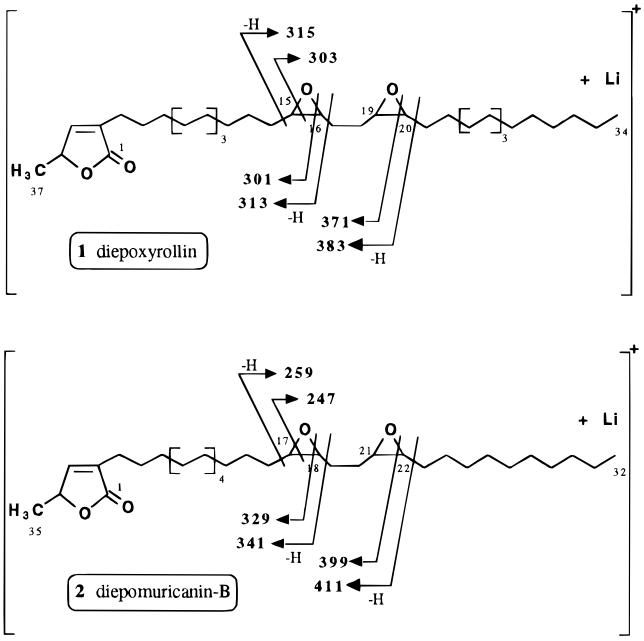

Figure 2. Li FABMS fragment ions of diepoxyrollin (**1**) and diepomuricanin B (**2**); CID, constant B/E linked-scan spectrum.

by collision-induced dissociations (CID) of $[M + Li]^+$ ions, using linked-scan analysis at constant B/E.[3] Thus, a series of significant fragment-ion peaks was observed, all of which retained lithium (Figure 2).

Besides loss of H_2O, leading to a fragment ion at *m/z* 563, fragmentation of the γ-lactone ring was recognized by loss of 28 (CO) and 44 (CO_2) mass units (*m/z* 553 and *m/z* 537, respectively). The presence of two pairs of peaks at *m/z* 301/313, *m/z* 371/383 suggested the position of epoxy rings to be at C-15/16 and C-19/20. Thus, the epoxy rings were each separated from one another by two methylenes. Two peaks at *m/z* 315 and 303 formed by fragmentation of the epoxide ring placed at C-15,16 and containing the methyl terminal were also observed (Figure 2).

The EIMS fragment ions of **1** showed a base peak at *m/z* 295 corresponding to the fragmentation of the first epoxide ring with regard to the lactone (between C-15 and C-16). On the other hand, a series of fragment ions between the lactone moiety and the epoxide ring with regard to the lactone was also observed.

Diepomuricanin B (**2**) was isolated as a white amorphous solid. Its molecular weight was determined by Li-FABMS at *m/z* $[M + Li]^+$ 553. HRCIMS (isobutane) from $[M^+]$ at *m/z* 546.2516, showed the molecular formula $C_{35}H_{62}O_4$ (calcd 546.2778).

All spectral data in ^{1}H NMR and ^{13}C NMR for **2** were very similar to those of **1**, which proved a structure type diepoxyacetogenin having a γ-lactone α,β-unsaturated without a hydroxyl at the C-4 position. In fact, the difference with diepoxyrollin (**1**) was deduced by examination of MS data. The experience of FABMS for **2** in presence of lithium, CID of $[M + Li]^+$ ions, using linked scan analysis at constant B/E, was employed. The characteristic fragmentation patterns obtained allowed us to confirm the presence of two epoxy rings and their location along the alkyl chain (between C-17/18 and C-21/22 (Figure 2). In the EIMS of **2**, the main peak corresponding to the fragmentation of the first epoxy ring (between C-17 and C-18) was at *m/z* 323. The series of fragmentation ions between the lactone moiety and the epoxy ring of **2** were also observed with two additional fragments at *m/z* 279 and *m/z* 293 (between C-15,16 and C-16,17, respectively) not found for **1**, confirming the location of epoxide rings.

Diepoxyrollin (**1**) and diepomuricanin B (**2**) are two new members of the diepoxyacetogenins class, formed by eight compounds so far.[9] In addition to **1** and **2**, three known diepoxyacetogenins, diepomuricanin A (**3**) and dieporeticanins 1 and 2 (**4**,**5**) were isolated and identified by ^{1}H-NMR, ^{13}C-NMR, and MS spectra, from *R. membranacea* seeds; those three compounds were previously reported from *Annona muricata* and *A. reticulata*;[3,4,9,10] as well as rollinone (**6**) and sylvaticin (**7**), previously isolated from *Rollinia papilionella*[5,6] and *R. sylvatica*,[7] respectively. Rollinone (or isorolliniastatin 1) (**6**) and all of the other described "iso"-acetogenins must not be considered as natural products but as artifacts of purification from classical mono- or bis-THF acetogenins hydroxylated in the 4 position as recently proved.[11−13] Translactonization of sylvaticin (4-hydroxyacetogenin) (**7**) into isosylvaticin (**7a**) (mixture of 2,4-*cis* and 2,4-*trans*), is accomplished quantitatively after treatment with an organic solution of 10% diethylamine during 18 h or after simple elution from Si gel column with a solvent mixture as hexane−EtOAc−diethylamine (6:3:1).

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Schmidt−Haensch Polartronic I at 589 nm. UV spectra were obtained in MeOH on a Unicam 1800 spectrometer. IR spectra were recorded in film on a Perkin-Elmer 257 unit. The ^{1}H-NMR and ^{13}C-NMR spectra (in $CDCl_3$ solution) were recorded using Bruker AC-200 (at 200 and 50 MHz, respectively) and Bruker AM-400 (at 400 and 100 MHz, respectively) systems. The EIMS was performed on a Nermag−Sidar spectrometer; and the FABMS, on a Kratos MS-80. TLC analysis was performed on 0.2-mm precoated plates (Si gel 60 F_{254}), and spots were detected by spraying with Kedde's reagent[8,11] and 50% H_2SO_4.

Plant Material. Seeds of *R. membranacea* TR. & PL. were collected from the region of Antioquia in San Luis, on the canyon of Rio Claro at 330 m altitude in Colombia. A voucher specimen (SM 1106) was deposited at the University of Antioquia in Medellin.

Extraction and Isolation. Extraction and fractionation were monitored by the brine shrimp test (BST).[14] Dried, powdered seeds (1900 g) from *R. membranacea* were extracted with MeOH by Soxhlet extraction. The MeOH extract (**E1**) showed important cytotoxicity with the BST (LC_{50} = 1.5 × 10^{-2} μg/mL). The concentrated

extract was fractionated by liquid−liquid partition between hexane and 5% aqueous MeOH to yield 467 g of a hexane-soluble fraction (**E2**) and an aqueous MeOH-soluble fraction (**E3**). **E3** was partially evaporated under reduced pressure and extracted with CH_2Cl_2 to yield the H_2O-soluble fraction (**E4**) and 145 g of the bioactive CH_2Cl_2-soluble fraction (**E5**). **E5** (100 g) (BST, $LC_{50} = 3 \times 10^{-3}$ μg/mL) was subjected to flash column chromatography over Si gel 60 and gradually eluted by mixtures of increasing polarity containing petroleum ether, CH_2Cl_2, and MeOH. Dieporeticanins 1 and 2 (**4**,**5**) (14 mg) were obtained as a mixture. Diepoxyrolline (**1**) (40 mg) and diepomuricanins A (**3**) (5 mg) and B {**2**} (10 mg) were all purified by column chromatography over Si gel 60 H column and eluted with C_6H_6−$(CH_3)_2$-CHOH (97:3). Rollinone (**6**) (750 mg) and sylvaticin (**7**) (250 mg) were purified by flash chromatography and eluted with cyclohexane−EtOAc (50:50).

Diepoxyrollin (1): $C_{37}H_{66}O_4$; $[\alpha]_D = +11°$ (*c* 0.85, $CHCl_3$); UV λ max nm 208; IR (film) ν max 2910, 2845, 1745 cm^{-1}; Li-FABMS (*m* − NBa) *m*/*z* 581 $[M + Li]^+$, 575 $[M + H]^+$, see Figure 2; EIMS *m*/*z* 295, 265, 251, 237, 223, 209, 195, 181, 167, 153, 139, 125, 111, 97; ^{1}H NMR (200 MHz, $CDCl_3$) and ^{13}C-NMR (50 MHz, $CDCl_3$) data, see Figure 1.

Diepomuricanin B (2): $C_{35}H_{62}O_4$; $[\alpha]_D = +10°$ (*c* 0.75, $CHCl_3$); UV λ max nm 208; IR (film) ν max 3400, 2910, 2845, 1745 cm^{-1}; Li-FABMS (*m* − NBa) *m*/*z* 553 $[M + Li]^+$, see Figure 2; EIMS *m*/*z* 323, 293, 279, 97; ^{1}H-NMR (200 MHz, $CDCl_3$) and ^{13}C-NMR (50 MHz, $CDCl_3$) data, see Figure 1.

Translactonization of 7 To Give 7a. Sylvaticin (**7**) (20 mg) was treated in an EtOAc−diethylamine (90:10) solution with Si gel at room temperature for 7 days to afford isosylvaticin (mixture of 2,4-*cis* and 2,4-*trans*) (**7a**) (quantitative yield): white wax; $C_{37}H_{66}O_8$; $[\alpha]_D$ +23° (*c* 0.3, MeOH); UV λ max nm (MeOH) 204; IR (film) ν max 3440, 2900, 2840, 1765, 1705 cm^{-1}; CIMS (CH_4) *m*/*z* $[MH]^+$ 639, $[MH - H_2O]^+$ 621, $[MH - 2\ H_2O]^+$ 603, $[MH - 3\ H_2O]^+$ 585, 467, 449, 431, 397, 379, 361, 339, 321, 311, 309, 293, 267 (100%); Li-FABMS (*m*-NBa) *m*/*z* $[MLi]^+$ 645; ^{1}H-NMR resonances were assigned by analysis of COSY 45 (200 MHz, $CDCl_3$) δ 4.36 (m, H-4 *cis*), 4.53 (m, H-4 *trans*), 3.85 (m, H-12,15,19,20,23), 3.42 (m, H-16,24), 3.09 and 3.06 (m, H-35b *cis* and *trans*), 3.00 and 3.02 (m, H-2 *cis* and *trans*), 2.73 and 2.70 (m, H-35a *cis* and *trans*), 2.21 (s, CH_3-37), 1.25−1.95 (m, CH_2-5 to -11, -13, -14, -17, -18, -21, -22, and -25−33), 0.88 (t, CH_3-34); ^{13}C-NMR multiplicities were determined by spin-echo correlated spectroscopy; heteronuclear correlation ^{1}H−^{13}C (XH CORR) was carried out (50 MHz, $CDCl_3$) δ 205.42 (C-36), 178.14 and 178.67 (C-1, *cis* and *trans*), 82.81 and 82.19 (C-23 and C-20), 81.74 and 79.16 (C-15 and C-12), 79.16 and 78.74 (C-4, *cis* and *trans*), 74.08 and 73.82 (C-16 and C-24), 72.03 (C-19), 44.02 and 43.57 (C-35, *cis* and *trans*), 35.39 (C-3 and C-17), 34.29 and 36.54 (C-2, *cis* and *trans*), 33.05, 32.95, and 32,20 (C-5, C-18, and C-25), 31.73 (C-32), 29.76 (C-37), 23.88−29.17 (C-6−C-11, C-13, -14, -21, -22 and C-26−C-31), 22.50 (C-33), and 13.93 (C-34).

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