Muconin and Mucoxin: Additional Nonclassical Bioactive Acetogenins from *Rollinia mucosa*

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The Annonaceous, acetogenins are a class of natural products that have excellent anticancer, antiinfective, and pesticidal properties.[1] While the pace of discovering unknown acetogenins is showing an upward trend,[1] most of the newly reported acetogenins, however, belong to several classical types usually having unsubstituted tetrahydrofuran (THF) rings.[1] Mucocin, the first tetrahydropyran (THP) ring-bearing acetogenin, was recently discovered in the bioactive leaf extracts of *Rollinia mucosa* (Jacq.) Baill. (Annonaceae)[2] and has, as well, a nonadjacent THF ring. We have now isolated another closely related acetogenin, muconin (**1**, Figure 1), that bears an unsubstituted THP ring along with an adjacent THF ring. **1** is the second THP-bearing acetogenin to be reported and further indicates the existence of a novel acetogenin type with THP ring(s) as the most salient structural feature. In addition, mucoxin (**2**, Figure 1) is also reported here as the first acetogenin that bears a hydroxylated THF ring.[3]

Both **1** and **2** were isolated[4] from the previously described leaf extract[5] by activity-directed open column fractionation using the brine shrimp lethality test[1] (BST) and, at later stages, by ^{1}H NMR-monitored purification using repetitive normal, and reversed-phase HPLC. The molecular formulas of **1** and **2**[4] apparently suggested that they were C_{37} acetogenins bearing two THF rings and three hydroxyls. The retention time of **2** on normal-phase HPLC, however, was much shorter than those typically observed for this type of acetogenins. Although the ^{1}H NMR spectra of **1** and **2** (Table 1)[6] showed the diagnostic peaks of the 2,4-disubstituted α,β-unsaturated γ-lactone terminal (**1** bears a 4-OH), which is common to most

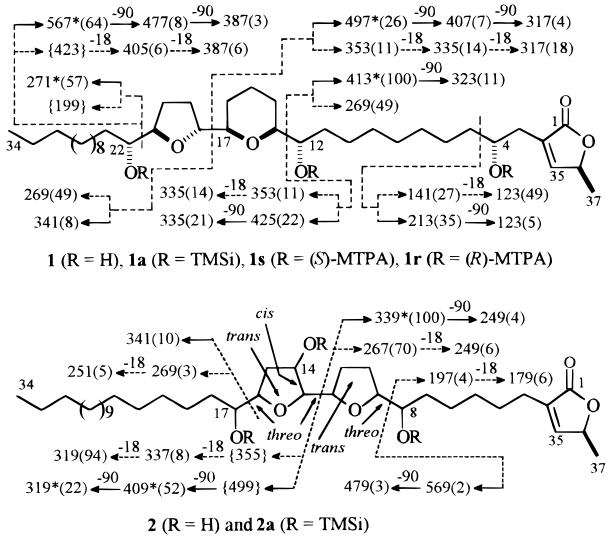

Figure 1. Chemical structures of **1**, **1a**, **1s**, **1r**, **2**, and **2a**. The EIMS *m/z* data of **1**, **1a**, **2**, and **2a** are shown [Numbers in parentheses are percent intensities; ions in brackets were not observed; dashed arrows are for **1** and **2**, and solid ones are for **1a** and **2a**; * ions confirmed by HREIMS].

acetogenins,[1] they also presented certain salient features. For **1**, the unusual δ values (3.16 and 3.31) of two protons were reminiscent of the THP oxymethines in mucocin.[2] For **2**, the presence of a well-defined pseudo triplet (1 H, δ 3.74, $J = 3$ Hz) was never encountered in our previous experiences with acetogenins. The above peaks were not lost to D_2O exchanges.

The presence of a THP ring in **1** was indicated by an intensive NOESY cross peak at δ 3.16/3.31 (later assigned as H-13/17) and the lack of the same cross peak in its regular, single-relayed, and double-relayed COSY spectra. The coexistence of a THF ring in **1** was required by its degree of unsaturation and was suggested by the ^{1}H and ^{13}C NMR data (Table 1). It was then deduced from the COSY spectrum that the THP ring was flanked on one side by a hydroxyl (OH-12) and, on the other, by a THF ring that was itself hydroxyl flanked. The position of the OH-12 was not proposed on the THP ring because no long-range ^{1}H−^{1}H coupling was observed between H-12 and additional oxygenated moieties, and the neighboring methylene protons of OH-12 did not show a split in their δ values.[7]

The above NMR-based structural hypothesis was ambiguous in the sequential placement of the THF/THP system, and the absolute coordinates of oxygenation were determined by the EIMS of **1** and its per-TMSi derivative (**1a**). The structure shown was further substantiated by the HREIMS of four diagnostic fragment ions of **1a** (Figure 1; also see the Supporting Information).

The assignment of the relative stereochemistry of **1**, except at C-17/18, was straightforward. The THF ring was *trans* because of the relatively large δ difference between the C-20 or C-21 methylene protons;[5] the THP ring was *cis* because of the positive NOESY correlation at H-13/17; and both of the rings were flanked by the hydroxyls in the *threo* fashion according to Born's rule.[2,8] The absolute stereochemistry of **1** was determined by the

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(1) For our most recent review on Annonaceous acetogenins, see: Zeng, L.; Ye, Q.; Oberlies. H. N.; Shi, G; Gu, Z. M.; He, K.; McLaughlin, J. L. *Nat. Prod. Rep.* **1996**, *29*, 249−310, and the references indicated therein.

(2) 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.

(3) Four acetogenins were previously reported as having hydroxylated THF rings (Nonfon, M.; Lieb, F.; Moeschler, H.; Wendisch, D. *Phytochemistry* **1990**, *29*, 1951−1954); these structures were erroneous and have been corrected.[1]

(4) **1**: 2.5 mg of white wax; (HRFABMS MH$^+$ *m/z* found 623.4900, calcd 623.4887, corresponding to $C_{37}H_{67}O_7$.) **2**: 1.8 mg of a colorless oil; (HRCIMS MH$^+$ *m/z* found 623.4899, calcd 623.4887, corresponding to $C_{37}H_{67}O_7$.)

(5) Shi, G.; Zeng, L.; Gu, Z. M.; MacDougal, J. M.; McLaughlin, J. L. *Heterocycles* **1995**, *41*, 1785−1796.

(6) The NMR data of the lactone portions were the same as previously reported.[1]

(7) The latter phenomenon should be otherwise prominent because the introduction of an hydroxyl group onto a THP (or THF) ring will induce significant differentiation of the chemical environment surrounding this hydroxyl. Mucocin[2] and **2** provide convenient examples for these situations.

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Table 1. Partial[6] ^{1}H and ^{13}C[a] NMR Data (δ, $CDCl_3$) for 1 and 2

		11	12	13	14, 15	16	17	18	19	20	21	22	23
1	^{1}H	1.42 m	3.43 m	3.16 m	1.25 m	1.35 m	3.31 m	3.90 m	1.65, 1.98 m	1.72, 1.98 m	3.80 m	3.38 m	1.40 m
	^{13}C	32−34	74.2	80.9	27−32	27−32	80.0	81.2	27−32	27−32	82.8	74.1	32−34

		7	8	9	10	11	12	13	14	15	16	17	18
2	^{1}H	1.40 m	3.42 m	3.95 m	1.91, 2.05 m	1.91, 2.05 m	4.31 m	3.71 t[b]	4.32 m	1.91, 2.35 m	4.04 m	3.53 m	1.50 m
	^{13}C	31−34	73.6	83.4	27−32	28−30	78.9	83.5	72.8	38.0	79.9	73.7	31−34

[a] Assignments assisted by HMQC and HMBC. [b] J = 3 Hz.

advanced Mosher ester method.[9,10] The (*R*)- and (*S*)-tri-MTPA esters (**1s** and **1r**) were prepared, and their ^{1}H δ values were assigned by the means of COSY and DQF-COSY.[11] The $\Delta\delta_H(S-R)$ values of H-11, H-13, and H-17 indicated an *S* configuration at C-12. The $\Delta\delta_H(S-R)$ values of H-21 and H-23 were both positive; nevertheless, the absolute configuration at C-22 could be determined as *R* according to the $\Delta\delta_H(S-R)$ value of H-23.[12] With the prior established relative stereochemistry in hand, the absolute configurations at C-13, C-17, C-18, and C-21 were deduced to be *S*, *R*, *R*, and *R*, respectively. At this point, the *threo* relationship at C-17/18 became self-evident.

The NMR data was again the key in solving the structure of **2**. The COSY spectrum of **2** showed that the pseudo triplet at δ 3.74 (later assigned as H-13) had only one apparent cross peak with the overlapped multiplets (2 H) centered at δ 4.31−4.32; therefore, the triplet H-13 must have coupled with both of the protons, which were oxymethines as indicated by their δ values. Hence, H-13 should assume the central position of a 1,2,3-trioxygenation pattern, sided by H-12 (δ 4.31) and H-14 (δ 4.32). It was also noticed that H-14 was coupled with a broad peak at δ 4.42 that was exchangeable with D_2O, and thus, H-14 was concluded to be a carbinyl oxymethine. The position of OH-14 was proposed on a rigid ring system rather than an open-ended hydrocarbon chain because of the large δ value difference of its neighboring methylene protons (2.35 for H-15a and 1.91 for H-15b).[7] H-16, an oxymethine at δ 4.04, was identified by tracing its COSY cross peaks to both H-15a and H-15b. At this point, a hydroxylated THF ring across C-13/16 was established. This perception was supported by the two new peaks at H-16/14 and H-15a/13 in the single-relayed COSY spectrum. The assignment of the second THF ring at C-9/12 was made possible by the H-12/9 cross peak (δ 4.31/3.95) in the double-relayed COSY spectrum. Finally, the two hydroxyls flanking the THF rings were clearly present.

The above structural proposal was supported by the EIMS of **2** and its per-TMSi derivative (**2a**, Figure 1), as well as the HREIMS of three diagnostic fragment ions (see the Supporting Information). These results also determined the oxygenation positions. It was noticed that an overwhelming percentage of the EI fragmentation took place at C-12/13, resulting in the concomitant intensity depression on other fragment ions. This phenomenon, while not typically observed in other adjacent bis-THF acetogenins, could be explained by an hypothesized dominant EI ion pathway for **2** and **2a**.[13]

Two THF flanking hydroxyls were obviously *threo* to the ring system.[8] Both THF rings were suggested to be *trans* because no NOESY correlation across the rings was detected.[14] The 3 Hz coupling constant between H-12 and H-13 indicated the freeze of rotation around the C-12/13 bond, which was a result of the likely intramolecular hydrogen bonding between OH-14 and the C-9/12 THF oxygen. A molecular model convinced us that in order for H-13 to maintain the 3 Hz coupling with both H-12 and H-14, the two THF rings must be *threo* to each other, and that OH-14 must be *cis* related to the C-13 side chain. The elucidation of the absolute stereochemistry of **2** was not attempted because of its total amount isolated.[4]

Compared to adriamicin, **1** showed potent and selective *in vitro* cytotoxicities against PACA-2 (pancreatic cancer) and MCF-7 (breast cancer) in a panel of six human solid tumor cell lines.[15] While the potency of **2** was generally lower than that of **1**, it was selectively inhibitory against MCF-7 in the same test run.[15] For the depletion of ATP as the mechanism of action for acetogenins, interested readers are referred to the original reports.[1]

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Supporting Information Available: UV, IR, HREIMS data, and 1D and 2D NMR spectra of **1** and **2** (8 pages).

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(8) Born, L.; Lieb, F. J.; Lorentzen, P.; Moeschler, H.; Nonfon, M.; Sollner, R.; Wendisch, D. *Planta Med.* **1990**, *56*, 312−316.

(9) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, Y. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096.

(10) The $\Delta\delta_{S-R}$ values of H-5, H-3a, H3b, H-35, H-36, and H-37 were found to be the same as those reported for all acetogenins with the same γ-lactone terminal, indicating the common configurations for the C-4 (*R*) and C-36 (*S*) stereocenters.[1,2,5]

(11) Because only 0.2 mg of the (*S*)-tri-MTPA ester of **1** was isolated, a DQFCOSY experiment was performed in order to minimize the diagonal peaks. Also, the solvent ($CDCl_3$) peak was suppressed by setting the transmitter frequency at δ 7.26. Some selective ^{1}H NMR δ values of **1s** and **1r** ($\Delta\delta_H(S-R)$) are as follows: H-11, 1.56, 1.60 (−0.04); H-12, 5.11, 5.04 (+0.07); H-13, 3.43, 3.40 (+0.03); H-17, 3.33, 3.26 (+0.07); H-18, 3.76, 3.92 (−0.16); H-21, 4.03, 3.98 (+0.05); H-22, 5.03, 5.01 (+0.02); H-23, 1.62, 1.45 (+0.17).

(12) Similar abnormalities in $\Delta\delta_H(S-R)$ values have been observed in several well-characterized adjacent bis-THF acetogenins, and certain theoretical explanations were suggested: Zhao, G. X.; Chao, J. F.; Zeng, L.; Rieser, M. J.; McLaughlin, J. L. *Bioorg. Med. Chem.* **1996**, *4*, 25−32.

(13) After the EI cleavage of **2** (or **2a**) at C-12/13, the left fragment (either a radical or radical cation) may lose two H_2O (or TMSiOH) units to form a conjugated diene system in which the radical at C-13 can be delocalized. Or, given the experimental temperature (*ca.* 250 °C) of the EI source, the thermal loss of H_2O (or TMSiOH) may take place before the cleavage at C-12/13, providing the kinetic driving force for this ion pathway. All the product and intermediate ions in the above proposed process were observed (Figure 1).

(14) While negative NOESY results were generally viewed as being indecisive, oxymethines on *cis* THF rings, however, always have shown strong NOESY correlation in our experience.

(15) The 7-day MTT *in vitro* cytotoxicity tests against six human tumor cell lines followed the standard protocols as previously described[5] and were performed at the Cell Culture Laboratory, Purdue Cancer Center. The ED_{50} (μg/mL) values of **1**, **2**, and adriamycin (as the standard positive control) are listed as follows (in the same compound order). A-498 (renal carcinoma): 1.8×10^{-1}, 8.4×10^{-1}, 2.6×10^{-3}. PC-3 (prostate adenocarcinoma): 5.8×10^{-1}, 3.1×10^{-1}, 4.2×10^{-3}. PACA-2 (pancreas carcinoma): 5.4×10^{-4}, 3.3×10^{-1}, 3.4×10^{-3}. A-549 (lung carcinoma): 4.5×10^{-3}; 3.6×10^{-2}, 2.5×10^{-3}. MCF-7 (breast carcinoma): 2.4×10^{-4}, 3.7×10^{-3}, 1.0×10^{-2}. HT-29 (colon adenocarcinoma): 3.9×10^{-1}, 6.1×10^{-1}, 1.8×10^{-2}.