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Aromin-A, an Annonaceous acetogenin from *Annona cherimola*

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Abstract

Chromatographic fractionation of a MeOH extract from the stems of *Annona cherimola* led to the isolation of a new non-adjacent, bis-THF ring acetogenin (one THF ring being at C-4 and the other at C-16), aromin-A (**1**) along with the known cytotoxic acetogenin squamocin (**2**). The structures of these isolates were established by means of mass spectrometry and spectroscopic techniques.

Keywords: Annona cherimola; Annonaceae; Aromin-A; Squamocin

1. Introduction

Since the discovery of uvaricin, the first Annonaceous acetogenin, in 1982 (Jolad et al., 1982), over 250 of these natural polyketides have been isolated (Kim et al., 1998). Annonaceous acetogenins have attracted much interest as potential anticancer agents in recent years (Alfonso et al., 1996). The mechanism of action of Annonaceous acetogenins is via inhibition of NADH: ubiquinone oxido-reductase (complex I) in the mitochondrial electron transport system and inhibition of NADH oxidase in the plasma membranes of tumor cells (Woo, Zeng, & McLaughlin, 1995). The structure–activity relationship for the Annonaceous acetogenins in mitochondria has been reported (Landolt et al., 1995). To our knowledge, two compounds, aromin and aromicin, with non-adjacent THF rings distant by more than four carbons have been isolated (Alfonso et al., 1996).

Guided by an in vitro assay testing for cytotoxicity of hepatoma cells, the fractionation procedures using modern separation methods resulted in the isolation of a new compound aromin-A (**1**), along with a known compound squamocin (**2**). Aromin-A (**1**), a C-20 epimer of aromin (**3**), reported in this paper, is a type of non-adjacent bis-THF ring acetogenin, with the two THF rings, beginning at C-4 and at C-16, separated by eight carbons and with C-9 bearing a carbonyl group. This type of Annonaceous acetogenin is reported only for the second time.

2. Results and discussion

From the methanolic extract of the fresh stems, **1** was obtained as a white waxy solid. The HRFABMS revealed an $[M+H–H_2O]^+$ ion at 575.4323 (calcd 575.4312), which indicated the molecular formula $C_{35}H_{60}O_7$. The spectral data of **1** are similar to those of **3** (Alfonso et al., 1996). Concerning moiety **a** (Scheme 1), the ^{1}H NMR spectrum of **1** showed resonances at δ 7.15 (H-33), 4.99 (H-34) and 1.40 (H-35) attributed to an α,β-unsaturated γ-lactone. The presence of this type of lactone was also shown in the ^{13}C NMR spectrum by the signals at δ 151.2 (C-33), 77.8 (C-34), 19.1 (C-35), 174.1 (C-1) and 130.6 (C-2). These assignments were confirmed by HMQC and HMBC spectra. The ^{13}C NMR spectrum of **1** displayed a signal at δ 209.2 for a ketone carbonyl group (revealed as being at C-9) and two signals at δ 49.1 and 43.8 (C-8

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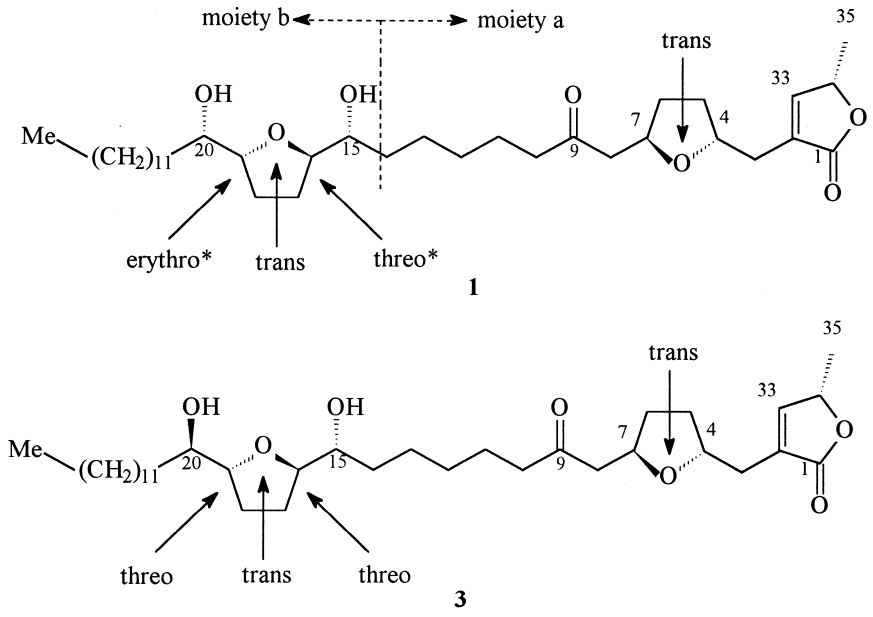


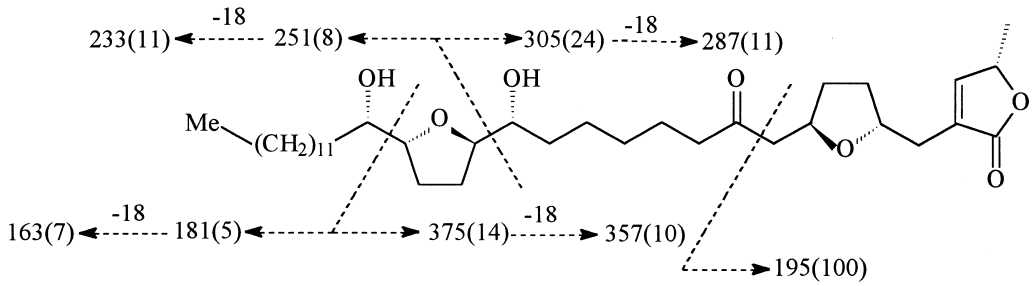
Scheme 1. Chemical structures of aromin-A (**1**) and aromin (**3**). Asterisks indicate that the sterechemistry may be interchangeable.

and C-10, respectively) corresponding to methylene carbons adjacent to the carbonyl. Resonances for methylene protons adjacent to a carbonyl were also present in the ^{1}H NMR spectrum at δ 2.42 (t, H-10), 2.38 (dd, H-8a) and 2.63 (dd, H-8b). The connectivities of these positions were confirmed by HMQC. Since the proton signals of one of the methylenes (H-8) of **1** were split, there had to be another functional group β to the carbonyl; the ^{1}H and ^{13}C NMR values (δ 3.84 and 74.1) were characteristic for an oxygenated function. On the other hand, signals for five protons overlapped in the narrow range of δ 2.35 to 2.45 in the ^{1}H NMR spectrum taken in $CDCl_3$. In this case, the ^{1}H and ^{13}C NMR values (δ 3.59 and 75.6) were characteristic of an oxygenated function.

A 4-OH group is often present in the Annonaceous acetogenins (Gu, Zhao, Oberlies, Zeng, & McLaughlin, 1995) and H-3a and H-3b give distinct ^{1}H NMR signals in $CDCl_3$ (Gu, Fang, Zeng, Wood, & McLaughlin, 1993) (the signals of 3a and 3b were overlapped for **1**). Also, the NMR values for the α,β-unsaturated γ-lactone were slightly different from those found in acetogenins bearing a 4-OH group (Born et al., 1990), giving evidence that the oxygenated function at C-4 is not a hydroxyl group. It was then deduced from the COSY spectrum that there was a THF ring present at C-4 to C-7 and a carbonyl group at C-9. Aromin-A (**1**) has been established to support the allocation of the THF ring and ketone present in moiety **a** by comparison with their ^{13}C NMR of aromin type acetogenins (Alfonso et al., 1996). The HMBC spectrum of **1** clearly displayed a cross-peak between C-9/H-10, C-9/H-8a, C-9/H-8b and C-7/H-8b which indicated the linkage of the THF ring and a carbonyl group at C-9.

The structure of moiety **b** of the molecule (Scheme 1) was readily determined, being a common feature among mono-THF ring Annonaceous acetogenines, i.e. a mono-THF ring flanked by two hydroxyl groups, with a long hydrocarbon chain on one side (McCloud, Smith, Chang, & Cassady, 1987; Li et al., 1990). The presence of two hydroxyl groups was obvious from the two resonances at δ 74.1 and 71.6 (C-15 or C-20) in the region of the hydroxyl-bearing carbons in the ^{13}C NMR spectrum, by two multiplets for two protons (H-15 or H-20) at δ 3.40 and 3.80 in the ^{1}H NMR spectrum, and by the loss of two molecules of H_2O from the molecular ion observed in the FABMS. The coupling between H-15/H-16 and H-19/H-20 in the COSY spectra of **1** proved that the hydroxyl groups were adjacent to the THF ring. The COSY spectra also showed couplings between H-16/H-17, H-17/H-18, H-18/H-19 as well as H-14/H-15 and H-20/H-21.

The relative stereochemical relationship between the chiral centers at C-15/C-16 and C-19/C-20 were defined as one *threo* and one *erythro* interchangeably, according to NMR studies of model compounds (Gu et al., 1993; Shi, Zeng, Gu, MacDougal, & McLaughlin, 1995). The *trans* configuration of the THF ring located in the moiety **b** was suggested by the close match of the ^{13}C NMR data (C-14 to C-21) with those of model compounds (Fujimoto et al., 1994) and by the chemical shift difference of 0.32 ppm between H-17a and H-17b, and H-18a and H-18b; this difference is close to 0.15 ppm in cases of a *cis* THF ring (Roepstorff, 1989; Gu et al., 1995). Similarly, for the



Scheme 2. Diagnostic EIMS peaks (m/z) for **1**. The percentage of fragment intensity was showed in perentheses.

THF ring located in moiety **a**, the difference of 0.34 ppm between H-5a and H-5b, and H-6a and H-6b, in the ^{1}H NMR spectra ($CDCl_3$) indicated that the stereochemistry of this THF ring is also *trans*. The stereochemical relationship at C-15–C-20 was deduced as *threo**/*trans*/*erythro** (*interchangeable). Based on the analysis of the ^{13}C NMR data, all of 15,20-dihydroxyl-16-mono-THF Annonaceous acetogenins are *threo* in C-15/C-16 (Zeng et al., 1996; Zafra-Polo, Figadere, Gallardo, Tormo, & Cortes, 1998). So, we predicted that **1** possessed 15,16-*threo* and 19,20-*erythro* configuration.

The molecular ion of **1** could be seen by FABMS, resulted in an $[M+H]^+$ ion at m/z 593 with two successive losses of H_2O (m/z 575 and 557) and with a strong ionized peak at m/z 615 $[M+Na]^+$. The placement of the THF ring flanked by two hydroxyl groups along with the aliphatic chain was determined by the EIMS of **1**, characteristic cleavages occur to give characteristic fragments (Scheme 2). From the above discussion, the structure of the new compound was deduced to be as depicted in Scheme 1, which we named aromin-A.

Aromin-A (**1**), showed cytotoxic activity against Hep. 2,2,15 (human hepatoma cell transfected HBV) and Hep. G_2 (human hepatoma cell) cells culture system with ED_{50} of 4.46 and 5.21 μg/ml, respectively. Squamocin (**2**), isolated from *A. reticulata* (Wu, Chang, Duh, & Wang, 1992; Chang, Wu, Duh, & Wang, 1993; Chang, Chen, Chiu, Wu, & Wu, 1998), *A. montana* (Wu, Chang, Chen, Liang, & Lee, 1994), *Rollinia mucosa* (Chen, Chang, Yen, & Wu, 1996) and *A. atemoya* (Chang, Chen, Chiu, Wu, & Wu, 1999) displayed potent cytoxic effect against Hep. 2,2,15, Hep. G2, KB, CCM2 at the concentration ED_{50} of 1.50×10^{-3}, 8.80×10^{-4}, 2.70×10^{-1} and 1.60×10^{-2} μg/ml, respectively (Chang et al., 1999).

3. Experimental

3.1. General

UV spectra (EtOH) were measured on a Hitachi U-2000 spectrophotometer. IR spectra (KBr) were measured on a Hitachi 260-30 spectrophotometer. ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz), HETCOR, COSY and DEPT spectra (all in $CDCl_3$) were obtained on a Varian NMR spectrometer (Unity Plus). Low-resolution FABMS and low-resolution EIMS were collected on a Joel JMS-SX/SX 102A mass spectrometer or Quattro GS/MS spectrometer having a direct inlet system. High-resolution EIMS were measured on a Joel JMS-HX 110 mass spectrometer. Si gel 60 (Merck, 230–400 mesh) was used for column chromatography; precoated Si gel plates (Merck, Kieselgel 60 F-254, 0.20 mm) were used for analytical TLC and precoated Si gel plated (Merck, Kieselgel 60 F-254, 0.50 mm) were used for preparative TLC. The spots were detected by spraying with Kedde's reagent or 50% H_2SO_4 and then heated on the hot plate.

3.2. Plant material

The stems of *A. cherimola* were collected from Chia-Yi, Taiwan in September 1996. A voucher specimen is deposited in the Graduate Institute of Natural Products, Kaohsiung Medical College, Kaohsiung, Taiwan.

3.3. Extraction and isolation

The fresh stems of *A. cherimola* M. (4.0 kg) were extracted repeatedly with methanol at room temperature. The combined methanol extracts were evaporated and partitioned to give $CHCl_3$, *n*-BuOH and aqueous solutions, respectively. The $CHCl_3$ layer was concentrated and chromatographed over silica gel using hexane/acetone as eluent to produce 25 fractions. Fraction 7 (1.03 g) eluted with EtOAc–hexane–acetone (10:10:1) was further separated and purified by silica gel column chromatography and preparative TLC ($CHCl_3$–MeOH, 10:1) to give squamocin (**2**) (5 mg). Fraction 16 (2.51 g) eluted with EtOAc–acetone (10:1) was further separated and purified by silica gel column chromatography and preparative TLC (EtOAc–acetone, 15:1) to give aromin-A (**1**) (6 mg).

Table 1
^{13}C and ^{1}H NMR spectroscopic data of Aromin-A (**1**). [a–e]Interchangeable within the same column

C/H	δ ^{13}C (100 MHz)	δ ^{1}H (400 MHz)
	1 ($CDCl_3$)	**1** ($CDCl_3$)
1	174.1	–
2	130.6	–
3a	31.1–31.9	2.36, m
3b		–
4	75.6	3.59, m
5a	31.1–31.9	1.22, m
5b		1.56, m
6a	31.1–31.9	1.22, m
6b		1.56, m
7	74.1	3.84, m
8a	49.1	2.38, dd (15.5, 4.0)
8b		2.63, dd (15.5, 9.0)
9	209.2	–
10	43.8	2.42, t (7.0)
11	29.3–29.7	1.3–1.8
12	25.2–25.6	1.3–1.8
13	25.2[a]	1.3–1.8
14	33.0[b]	1.39, m
15	74.1[c]	3.40, m
16	83.1[d]	3.79, m
17a	28.6[e]	1.66, m
17b		1.98, m
18a	25.2[e]	1.66, m
18b		1.98, m
19	82.2[d]	3.79, m
20	71.6[c]	3.80, m
21	32.5[b]	1.39, m
22	26.0[a]	1.3–1.8
23	29.3–29.7	1.3–1.8
24–30	23.3–31.9	1.3–1.8
31	22.7	1.3–1.8
32	14.1	0.88, t (7.0)
33	151.2	7.15, br. s
34	77.8	4.99, qddd (6.5, 1.5, 1.5, 1.5)
35	19.1	1.40, d (6.5)

3.4. Aromin-A (*1*)

White waxy solid (6 mg), m.p. 55–57°C. $[\alpha]_D^{24}$ +23.0° (*c* 0.05, $CHCl_3$). UV λ_{max} (EtOH) nm: 230. IR ν_{max} (neat) cm^{-1}: 3450, 1750, 1710. HRFABMS: $[MH–H_2O]^+$ *m/z* 575.4327 (calcd for $C_{35}H_{60}O_7$, 575.4312). FABMS: *m/z* 575 $[MH–H_2O]^+$ (6), 557 $[MH–2H_2O]^+$ (4). EIMS: Scheme 2. ^{1}H NMR and ^{13}C NMR: Table 1.

3.5. Squamocin (*2*)

Waxy solid (5 mg), $[\alpha]_D^{24}$ +20.0° (*c* 0.05, $CHCl_3$). Identified by direct comparison with data of the authenthic sample (TLC, UV, IR, EIMS, FABMS, and ^{1}H, ^{13}C NMR) and with the literature (Wu et al., 1992, 1994; Chang et al., 1993, 1998, 1999; Chen et al., 1996).

3.6. Bioassays

The cytotoxicity assay were carried out according to procedures described in the literature (Doong, Tsai, Schinazi, Liotta, & Cheng, 1991; Elliott, & Auersperg, 1993).

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