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Aromin and Aromicin, Two New Bioactive Annonaceous Acetogenins, Possessing an Unusual Bis-THF Ring Structure, from *Xylopia aromatica* (Annonaceae)

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Abstract: Our continuing activity-directed search for new bioactive compounds led to the isolation of two new, unusual, acetogenins, aromin (**1**) and aromicin (**2**), from the stem bark of *Xylopia aromatica* (Annonaceae). Both represent a new type of non-adjacent, bis-THF ring acetogenins (one THF ring being at C-4 to C-7 and the other at C-16 to C-19). Both show significant cytotoxicities among six human tumor cell lines; however, the activity is notably reduced compared to other non-adjacent bis-THF ring acetogenins. The structures of **1** and **2** were elucidated by spectroscopic methods and by derivatization.

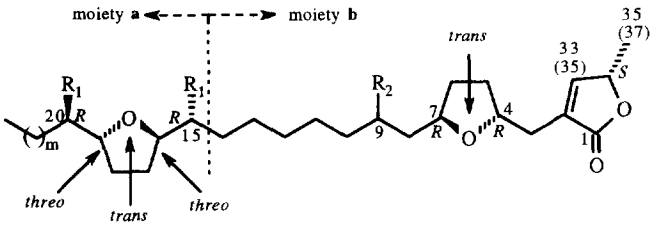
INTRODUCTION

Since the discovery of uvaricin, the first Annonaceous acetogenin, in 1982,[1] approximately 200 of these natural polyketides have been isolated. The interest shown for this class of compounds is justified by their highly potent and diverse bioactivities,[2] including promising cytotoxicities and *in vivo* antitumor effects, due to inhibition of NADH-ubiquinone oxido-reductase, which is an essential enzyme at complex I of oxidative phosphorylation in mitochondria,[3] and due to inhibition of the NADH oxidase that is elevated in plasma membranes of cancerous cells;[4] the consequence of both actions is depletion of intracellular ATP levels.

Structure-activity relationships for the Annonaceous acetogenins in mitochondria have been reported,[5] and it is known that the bis-tetrahydrofuran (THF) ring acetogenins are more potent than the mono-THF ring acetogenins, regardless if the THF rings are adjacent or non-adjacent. However, so far only compounds with non-adjacent THF rings distant by no more than four carbons have been isolated. Aromin (**1**) and aromicin (**2**), reported in this paper, present a completely new type of non-adjacent bis-THF ring acetogenins, 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. The influence of these structural features reduces the level of activity to that of the mono-THF ring compounds. Reduction of the C-9 carbonyl to the racemic carbinol increases the activity but not to the level of potency anticipated for the usual non-adjacent bis-THF compounds.

The genus *Xylopia* (Annonaceae) is composed of ca. 150 species distributed worldwide in the tropics.[6] *X. aromatica* (Mart.) Lam. is a tree growing in the tropical areas of America. Our sample was collected in Venezuela in the state of Amazonas. The isolation of **1** and **2**, directed by a test for lethality to brine shrimp (BST),[7,8] extends the structural diversity of the acetogenins synthesized by that species; several mono-THF ring, adjacent

bis-THF ring, and non-THF ring acetogenins have been previously isolated, many of them for the first time.[9-12] These have diverse structural features, representing C35 as well as C37 compounds, including double bonds, carbonyl substitutions, 1,2-vicinal diols, and the α,β-unsaturated γ-lactone as well as ketolactone ring systems.[2]



Compound	m	R_1	R_2
1	11	OH	=O
2	13	OH	=O
1a	11	OTMSi	=O
2a	13	OTMSi	=O
1b	11	OAc	=O
2b	13	OAc	=O
1c	11	OH	OH
1d	11	OTMSi	OTMSi
1r	11	O-(*R*)-MTPA	=O
1s	11	O-(*S*)-MTPA	=O
2r	13	O-(*R*)-MTPA	=O
2s	13	O-(*S*)-MTPA	=O

Fig. 1. Chemical structures of aromin (**1**), aromicin (**2**), and their derivatives.

RESULTS AND DISCUSSION

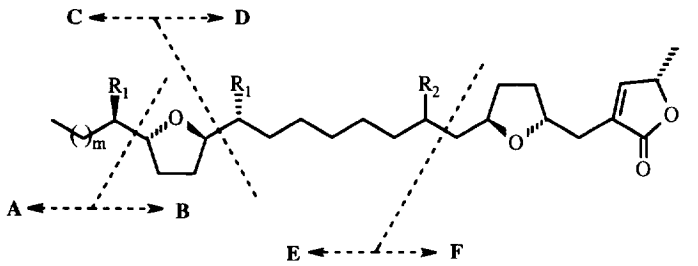
From the ethanolic extract of the bark, **1** and **2** were obtained by activity-directed fractionation (see Experimental) as waxy white amorphous solids. From the MS (which revealed a difference of 28 mass units), the similarities of the NMR spectra, and the fact that the ^{13}C-NMR spectrum of **2** contained two more signals than that of **1** (Table 1), it was obvious that **2** differed from **1** only by the length of the hydrocarbon chain. The structure of moiety **a** of the molecule (Figure 1) was easily determined, being a common feature among mono-THF ring Annonaceous acetogenins, i.e., a mono-THF ring flanked by two hydroxyl groups, with a long hydrocarbon chain on one side.[13,14] The presence of two hydroxyl groups was obvious from two resonances at δ 74.1 and 73.7 (C-15 and C-20) in the region of hydroxyl-bearing carbons in the ^{13}C-NMR spectrum, by a multiplet for two protons (H-15 and H-20) at δ 3.40 in the ^{1}H-NMR spectrum, by analysis of the EIMS of the TMSi derivatives (**1a** and **2a**), by formation of the diacetate derivative of **1** (**1b**), and by loss of two molecules of H_2O in the CIMS. All of the signals of **1b** taken in C_6D_6 were well separated (in opposition to the spectrum taken in $CDCl_3$), and the multiplets corresponding to H-15 and H-20, bearing the acetyl groups, were shifted downfield by 1.74 ppm in comparison to **1**. The couplings between H-15/H-16 and H-19/H-20 in the COSY spectra of **1** and **1b** proved that the hydroxyl groups (or the acetyl groups in **1b**) 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

Table 1. ^{13}C and ^{1}H NMR Data of Aromin (**1**), Aromicin (**2**) and Aromin-15,20 diacetate (**1b**).

C / H	δ ^{13}C (125 MHz)		δ ^{1}H (500 MHz)			
	1 ($CDCl_3$)	**2** ($CDCl_3$)	**1** ($CDCl_3$)	**2** ($CDCl_3$)	**1** (C_6D_6)	**1b** (C_6D_6)
1	174.2	174.2	-	-	-	-
2	130.6	130.6	-	-	-	-
3a	31.1-31.9	31.0-31.9	2.36, m	2.36, m	2.22, dddd (16.0;3.5;1.5;1.5)	2.22, dddd (16.0;3.5;1.5;1.5)
3b					2.33, dddd (16.0,9.0,1.5,1.5)	2.33, dddd (16.0;9.0;1.5;1.5)
4	75.6	75.6	3.59, m	3.59, m	3.34, m	3.34, m
5a	31.1-31.9	31.0-31.9	1.22, m	1.23, m	0.98, m	0.98, m
5b			1.56, m	1.56, m	1.17, m	1.17, m
6a	31.1-31.9	31.0-31.9	1.22, m	1.23, m	0.93, m	0.93, m
6b			1.56, m	1.56, m	1.20, m	1.20, m
7	74.1	74.0	3.84, m	3.85, m	3.67, m	3.66, m
8a	49.1	49.1	2.38, dd (15.5;4.0)	2.38, dd (15.5;4.0)	1.93, dd (15.5;3.5)	1.93, dd (15.5;3.5)
8b			2.63, dd (15.5;9.0)	2.63, dd (15.5;9.0)	2.37, dd (15.5;9.0)	2.37, dd (15.5;9.0)
9	209.3	209.3	-	-	-	-
10	43.8	43.8	2.42, t (7.0)	2.42, t (7.0)	2.15, m	2.08,t (7.0)
11	29.3-29.7	23.3-31.9	1.3-1.8	1.3-1.8	1.6, m	0.94-1.76
12	25.2-25.6	29.3-29.7	"	"	0.94-1.76	"
13	25.2[a]	25.6[a]	"	"	"	"
14	33.5[b]	33.5[b]	1.39, m	1.39, m	1.25-1.53	1.5[a]
15	74.1[c]	73.7[c]	3.40, m	3.40, m	3.31, m	5.05 m
16	82.7[d]	82.7[d]	3.79, m	3.79, m	3.65, m	3.97, m
17a	28.7[e]	28.7	1.66, m	1.66, m	1.40, m	1.41, m
17b			1.98, m	1.98, m	1.59, m	1.65, m
18a	28.7[e]	28.7	1.98, m	1.66, m	1.40, m	1.41, m
18b				1.98, m	1.59, m	1.65, m
19	82.5[d]	82.5[d]	3.79, m	3.79, m	3.65, m	3.97 m
20	73.7[c]	73.7[c]	3.40, m	3.40, m	3.31, m	5.05, m
21	33.3[b]	23.3[b]	1.39, m	1.39, m	1.25-1.53	1.7[a]
22	25.6[a]	25.2[a]	1.3-1.8	1.3-1.8	0.94-1.76	0.97-1.76
23	29.3-29.7	29.3-29.7	"	"	"	"
24-30	23.3-31.9	23.3-31.9	"	"	"	"
31	22.7	"	"	"	"	"
32	14.1	"	0.88, t (7.0)	"	0.91, t (7.0)	0.91, t (7.0)
33	151.3	22.7	7.15, bs	"	6.66, m	6.65, ddd (1.5;1.5;1.5)
34	77.8	14.1	4.99, qddd (6.5;1.5;1.5;1.5)	0.88, t (7.0)	4.50, qddd (6.5;1.5;1.5;1.5)	4.51, qddd (6.5;1.5;1.5;1.5)
35	19.1	151.3	1.40, d (6.5)	7.15, bs	1.01, d (7.0)	1.02, d (6.5)
36	-	77.8	-	4.98, qddd (6.5;1.5;1.5;1.5)	-	-
37	-	19.1	-	1.40, d (6.5)	-	-
15-OAc	-	-	-	-	-	1.82, s[b]
20-OAc	-	-	-	-	-	1.83, s[b]

[a to e] interchangeable within the same column

and H-20/H-21. The placement of the THF ring flanked by two hydroxyl groups along the aliphatic chain was determined by the EIMS of the di-TMSi derivatives, **1a** and **2a**; characteristic cleavages occur to give fragments A to D (Figure 2).



Compound	R_1	R_2	$M(TMSi)_2^+$ (**1a**, **2a**) or $M(TMSi)_3^+$ (**1d**)	A	B	C	D	E	F
1a (m=11)	OTMSi	=O	646 (1)[a]	271 (22) 181 (1)[a]	465 (25) 375 (6)[a]	341 (9) 251 (1)[a]	395 (100) 305 (1)[a]		195 (49)
2a (m=13)	OTMSi	=O	764 (1) 674 (1)[a]	299 (24) 209 (3)[a]	465 (26) 375 (5)[a]	369 (10) 279 (1)[a]	395 (100) 305 (2)[a]		195 (45)
1d (m=11)	OTMSi	OTMSi	810 (6) 720 (5)[a] 630 (4)[b] 540 (11)[c]	271 (31) 181 (2)[a]	530 (24) 449 (9)[a] 359 (9)[b]	341 (9)	469 (100) 379 (30)[a] 289 (5)[b]	615 (6) 345 (1)[c]	195 (33)

Fig. 2. EIMS data of **1a**, **2a** and **1d**; [a] loss of 1 TMSiOH (*m/z* 90),[b] loss of 2 TMSiOH,[c] loss of 3 TMSiOH.

Concerning moiety **b** (Figure 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 signals at δ 151.3 (C-33), 77.8 (C-34), 19.1 (C-35), 174.2 (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 209.3 for a carbonyl group (revealed as being at C-9) and two signals at δ 49.1 and 43.8 (respectively, C-8 and C-10) 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** was 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 were overlapped in the narrow range of δ 2.35 to 2.45 in the ^{1}H-NMR spectrum taken in $CDCl_3$; although the presence of different resonances and their couplings could be proven by decoupling experiments, the spectrum was clarified in C_6D_6 and revealed, in particular, two distinct peaks for H-3a and H-3b (Table 1), demonstrating a substitution at C-4. In this case, also, the ^{1}H- and ^{13}C-NMR values (δ 3.59 and 75.6) were characteristic for an oxygenated function.

A 4-OH group is often present in the Annonaceous acetogenins;[2] then, however, H-3a and H-3b give separated NMR signals in $CDCl_3$[18] (these were overlapped for **1** and **2**). Also, the NMR values for the α,β-unsaturated γ-lactone were slightly different from those found in acetogenins bearing a 4-OH group,[15] giving evidence that the oxygenated function at C-4 is not a hydroxyl group. It was then deduced from the COSY and the single and double relayed COSY NMR spectra that there was a second THF ring present at C-4 to C-7 and a carbonyl group at C-9. The HMBC spectrum of **1** clearly displayed cross-peaks between C-11/H-10, C-9/H-8a, C-9/H-8b, C-9/H-10, C-7/H-8b, C-6/H-8b, C-5/H-3 and C-4/H-3. Since the molecular formula of **1** was established to be $C_{35}H_{60}O_7$ by HRCIMS on the basis of the diacetate (**1b**), which gave a molecular ion (MH^+) at *m/z* 677.4636 (calcd. for $C_{35}H_{58}O_7{\cdot}2CH_3CO$, 677.4629), the unsaturation number of six calculated for **1** confirmed the structure as proposed in Figure 1. The molecular ion of **1** could not be seen by CIMS or FABMS, but PDMS (plasma desorption MS)[16] resulted in a MH^+ of *m/z* 593 with two successive losses of H_2O (*m/z* 575 and 557) and with two strong signals at *m/z* 615 (MNa^+) and 631 (MK^+). Thus, the diacetate derivatives, **1b** and **2b**, had to be used to determine the exact masses. The important peak at *m/z* 195 found in the EIMS spectra of **1a** and **2a** was attributed to fragment F resulting from a cleavage β to the THF ring. To prove this assignment, **1** was reduced using $NaBH_4$ to give **1c**. The tri-TMSi derivative (**1d**) of **1c** still displayed this signal at *m/z* 195, while the values of fragments B, D and E, containing the third TMSi group, increased accordingly and further confirmed the proposed structure.

The relative stereochemical relationship between the chiral centers at C-15/C-16 and C-19/C-20 were both defined as *threo*, according to NMR studies of model compounds.[17,18] The *trans* configuration of the THF ring located in the moiety **a** was suggested by the close match of the ^{13}C-NMR data (C-14 to C-21) with those of model compounds[18] 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.[2,19] Similarly, for the THF ring located in moiety **b**, 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*. Moreover, no cross-peak at H-4/H-7, or at H-16/H-19, could be found in the NOESY spectrum of **1** (a cross-peak should be present in case of a *cis* THF ring). The absolute stereochemistries at C-15 and C-20 were determined by preparing the di-(*R*)- and -(*S*)-methoxytrifluoromethylphenyl acetic acid (MTPA) esters (Mosher esters) of **1** (**1r** and **1s**) and **2** (**2r** and **2s**). This technique analyzes the $\Delta\delta_H$ (δ_S-δ_R) on both sides of the chiral center.[20,21] Assignments of the signals of **1r**, **1s**, **2r** and **2s** were made using COSY spectra. The positive value of $\Delta\delta_H$ for H-14 and H-21 and the negative value of $\Delta\delta_H$ for H-16 to H-19 (Table 2), showed that H-14 and H-21 are relatively less shielded and H-16 to H-19 are relatively more shielded in the *S* MTPA esters than in the *R* MTPA esters; these data defined the absolute configurations at C-15 and C-20 as *R*. To propose the stereochemistries at C-4 and C-34 (C-36 for **2**), the CD curve of **1** was compared with those of several acetogenins previously proven to have 4*R*,34*S* (or 4*R*,36*S* in cases of C37 compounds) configurations. Since the stereochemistry of all known acetogenins substituted at C-4 is 4*R*,34*S* (or 4*R*,36*S*) and since **1** and **2** differ only by the length of the carbon chain, **2** was assumed to have the same absolute stereochemistry. Based on this information, the complete absolute stereochemistries of **1** and **2** were assigned as 4*R*, 7*R*, 15*R*, 16*R*, 19*R*, 20*R* and 34*S* (36*S* for **2**), the same as with all the acetogenins isolated so far from *X. aromatica*.[9-11]

Table 2. Chemical Shifts of Relevant Protons of the (*R*)- and (*S*)-MTPA esters **1r**, **1s**, **2r** and **2s**.

MTPA ester	H-14	H-15	H-16	H-17ab	H-18ab	H-19	H-20	H-21
1r	1.55	5.02	4.00	1.58 1.92	1.58 1.92	4.00	5.02	1.55
1s	1.57	4.94	3.92	1.38 1.68	1.38 1.68	3.92	4.94	1.57
$\Delta\delta_H(S\text{-}R)$	+ 0.02	-	- 0.08	- 0.20 - 0.24	- 0.20 - 0.24	- 0.08	-	+ 0.02
carbinol configuration	15*R*				20*R*			
2r	1.55	5.02	4.00	1.58 1.92	1.58 1.92	4.00	5.02	1.55
2s	1.58	4.94	3.92	1.38 1.66	1.38 1.66	3.92	4.94	1.58
$\Delta\delta_H(S\text{-}R)$	+ 0.03	-	- 0.08	- 0.20 - 0.26	- 0.20 - 0.26	- 0.08	-	+ 0.03
carbinol configuration	15*R*				20*R*			

Bioactivity data obtained with **1** and **2** are summarized in Table 3. Both acetogenins were toxic to the brine shrimp larvae and showed significant cytotoxicities against the six human solid tumor cell lines tested *in vitro*. No significant difference in activity could be observed between **1** and **2**. The activity of the reduced derivative (**1c**) was increased by one or two orders of magnitude in comparison to the original compound **1**; this observation is in agreement with previous reported data showing that three free hydroxyl groups are optimum for the potency of the acetogenins.[5,13,22]

Table 3. Brine Shrimp Lethality and Cytotoxicities in Human Solid Tumor Cell Lines for **1**, **2** and **1c**.

Compound	BST[a] LC_{50} µg/ml	A-549[b] ED_{50} µg/ml	MCF-7[c] ED_{50} µg/ml	HT-29[d] ED_{50} µg/ml	A-498[e] ED_{50} µg/ml	PC-3[f] ED_{50} µg/ml	PACA-2[g] ED_{50} µg/ml
1[h]	31.86	$2.89.10^{-1}$	1.22	1.18	$7.50.10^{-1}$	$5.98.10^{-1}$	$8.99.10^{-1}$
2[h]	10.28	$2.17.10^{-1}$	$4.24.10^{-1}$	1.09	$2.61.10^{-1}$	$3.01.10^{-1}$	$2.25.10^{-1}$
adriamycin[h,j]	-	$5.20.10^{-2}$	$5.75.10^{-1}$	$6.51.10^{-2}$	$3.42.10^{-2}$	$1.21.10^{-1}$	$2.55.10^{-2}$
1c[i]	> 50	$4.67.10^{-3}$	$1.84.10^{-2}$	$2.30.10^{-2}$	$2.09.10^{-2}$	$5.68.10^{-2}$	$1.45.10^{-1}$
adriamycin[i,j]	-	$8.87.10^{-3}$	$4.05.10^{-1}$	$2.73.10^{-2}$	$3.79.10^{-2}$	$4.56.10^{-2}$	$2.77.10^{-3}$

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[a]Brine shrimp lethality test.[7,8] [b]Lung carcinoma.[23] [c]Breast carcinoma.[23] [d]Colon Adenocarcinoma.[25] [e]Renal carcinoma.[24] [f]Prostate adenocarcinoma.[26] [g]Pancreas carcinoma.[27] [h,i]Determined in the same run. [j]Adriamycin was used as a positive control.

1 and **2** are non-adjacent bis-THF ring acetogenins of a completely new type, since the two THF rings are separated by eight carbons and in the common type they are separated by only four carbons.[2] Landolt *et al.* studied structure-activity relationships by measuring the oxygen uptake by rat liver mitochondrial suspensions and showed that bis-THF ring acetogenins are at least ten times more potent than mono-THF ring acetogenins, regardless if the rings are adjacent or non-adjacent.[5] However, the activities of **1** and **2** are comparable to those of the mono-THF ring compounds. On the other hand, Hopp *et al.* recently isolated squamotacin, a compound identical to bullatacin but with the two adjacent THF rings shifted by two carbons toward the lactone, and it

showed high selectivity for PC-3 cells (prostate adenocarcinoma) but had decreased activity for other cell lines.[28] Also, an acetogenin identical to asimicin but with the two THF rings shifted by four carbons toward the lactone has been isolated recently, and its activity is reduced to that of the mono-THF ring compounds.[29] These results suggest that two important factors for the potency of acetogenins are: a) two THF rings either adjacent or non-adjacent with a limited number of carbons between them and b) the adjacent bis-THF ring system may be shifted along the hydrocarbon chain toward the lactone by two, or maybe three carbons, but about eight methylene carbons should intervene in order to maintain a high level of nonselective potency.

EXPERIMENTAL

General Experimental Procedures

Mps were determined on a Fisher-Johns melting point apparatus and are uncorrected. UV spectra were taken on a Beckman DU-7 spectrophotometer. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. CD spectra were performed on a JASCO Model J600 Circular Dichroism Spectropolarimeter. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian VXR-500S (^{1}H at 500 MHz, ^{13}C at 125.75 MHz) spectrometer with proton signals referenced to TMS and carbon resonances referenced to $CDCl_3$. Low resolution CIMS and EIMS data were collected on a Finnigan 4000 spectrometer. FABMS, EIMS for TMSi derivatives, and HRFAB through peak matching were performed on a Kratos MS50 mass spectrometer. PDMS was achieved on a Bioion 20R (Bioion KB, Uppsala, Sweden) plasma desorption mass spectrometer; **1** was applied to a nitrocellulose-coated mylar target and allowed to dry before being put into the mass spectrometer.

Chromatography

HPLC was carried out on a Rainin HPLC instrument using the Dynamax software system and a RP-18 Si gel (8 μm) column (21.4 x 250 mm) equipped with a Rainin UV-1 detector set at 223 nm. Analytical TLC was performed on Si gel plates developed with $CHCl_3$-MeOH (12:1) and hexane-Me_2CO (2:1) and visualized with 5% phosphomolybdic acid in EtOH and heating.

Plant material

Bark of *Xylopia aromatica* was collected in the Estado Amazonas (Venezuela). The material was authenticated by Stephen Tillett at the Herbario Ovalles. A voucher specimen of the bark is deposited in the Herbario Ovalles, Universidad Central de Venezuela.

Bioassays

The extracts, fractions, and isolated compounds were evaluated for lethality to brine shrimp larvae (BST).[7,8] Cytotoxicities against six human solid tumor cell lines were measured in 7-day MTT tests at the Cell Culture Laboratory, Purdue Cancer Center, against cells of A-549 lung carcinoma,[23] MCF-7 breast carcinoma,[24] HT-29 colon adenocarcinoma,[25] A-498 renal carcinoma,[24] PC-3 prostate adenocarcinoma[26] and PACA-2 pancreas carcinoma,[27] using adriamycin as a positive control.

Extraction and isolation

The dried pulverized bark (4 kg) was extracted with EtOH (F001). The F001 residue was partitioned between H_2O (F002) and $CHCl_3$ (F003), and the residue of F003 was partitioned between hexane (F006) and aqueous 90 % MeOH (F005). F005 (60 g) was subjected to column chromatography over Si gel (2 kg) eluted with a gradient of hexane/EtOAc/MeOH. Fractions $F_1$1 to $F_1$92 were collected and pooled according to their similar TLC patterns. Bioactivities in the BST showed different active pools; isolations from some others are described elsewhere.[9-13] The active pool P4 (8 g) was further resolved on another Si gel (300 g) column eluted with 1.5 % MeOH in $CHCl_3$. Fractions $F_2$1 to $F_2$64 were collected into six pools on the basis of similar TLC patterns. One of the resulting active pools was submitted to a third Si gel column and eluted with a gradient of hexane/Me_2CO. **1** and **2**, contained in fractions 7 to 9 (out of 13), were purified by HPLC on RP18 using a gradient of MeCN/H_2O (flow rate 10 ml/min.).

TMSi derivatives

Approximately 0.3 mg of **1**, **1c** and **2** were placed in a 100 µl conical reaction vial and dried in a vacuum desiccator over P_2O_5 for 24 h. The samples were treated with 2 µl of pyridine and 20 µl of N,O-bis-(trimethylsilyl)-acetamide (BSA) and heated at 70° for 30 min. to yield the respective di- or tri-TMSi derivatives (**1a**, **1d** and **2a**).

Aromin- and aromicin-15,20-diacetates

To 1 to 1.5 mg of **1** and **2**, dissolved in 0.5 ml of pyridine, were added 0.5 ml of acetic anhydride. The mixture was set at room temperature for 4-5 h and dried overnight to yield **1b** and **2b**.

*Aromin-15,20-diacetate (**1b**).* Colorless wax. CIMS: *m/z* 677 (MH^+), 617 (MH^+-AcOH), 209, 195; HR-CIMS: 677.4636 (MH^+) (calcd 677.4629); ^{1}H-NMR (C_6D_6): Table 1.

*Aromicin-15,20-diacetate (**2b**).* Colorless wax. FAB (glycerol): *m/z* 705 (MH^+), 645 (MH^+-AcOH), 627 (MH^+-AcOH-H_2O); HR-FAB: 705.4920 (MH^+) (calcd 705.4942).

*Reduction of **1***

3 mg of **1** were dissolved in 0.5 ml of MeOH and 0.05 ml of THF and treated with 25 µl of $NaBH_4$ (0.5 M in 2-methoxyethylether). After 10 min. in an ice-bath, the reaction mixture was placed 3 h at room temperature. Diluted HCl (pH 5) was added, and the reduced aromin (**1c**) was extracted with $CHCl_3$.

*Aromin-9-hydroxyl (**1c**).* Colorless oil. FAB (glycerol): *m/z* 595 (MH^+), 577 (MH^+-H_2O), 559 (MH^+-2H_2O); EIMS of tri-TMSi derivative (**1d**): Figure 2.

Mosher esters

The isolated acetogenin (0.5-1 mg of **1** and **2** in 0.3 ml of CH_2Cl_2) was treated with 0.2 ml of pyridine, 0.5 mg of 4-(dimethylamino)pyridine and 25 mg of (*R*)- or (*S*)-(-)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride. The mixture was stirred at room temperature for at least 4 h, passed through a diposable pipet (0.6 x 6 cm) filled with Si gel and eluted with 3 ml of CH_2Cl_2. The residue was dissolved in CH_2Cl_2, washed with 5 ml of 1% $NaHCO_3$ and 2 x 5 ml of H_2O. The organic layer contained the MTPA ester. The (*R*)-(-)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride gave the *S*-Mosher esters **1s** and **2s**. The (*S*)-(-)-α-methoxy-α-

(trifluoromethyl)-phenylacetyl chloride gave the *R*-Mosher esters **1r** and **2r.** Assignments of the relevant protons: Table 2.

Aromin (1)

White waxy solid (20 mg); mp 48-49°; $[\alpha]_D^{22}$: + 10.3° (c = 0.25, $CHCl_3$); $UV_{\lambda max}$ ($CHCl_3$) 230 (ε = 965); CD (MeOH) $[\theta]_{235.8}$ -1514.57, $[\theta]_{277.0}$ -810.15; CIMS *m/z* 575 (MH^+-H_2O), 557 (MH^+-$2H_2O$). EIMS of TMSi derivative: Figure 2; ^{1}H-NMR: Table 1; ^{13}C-NMR: Table 1.

Aromicin (2)

White waxy solid (6 mg); mp 61-63°; $[\alpha]_D^{22}$: + 9.9° (c = 0.43, $CHCl_3$); $UV_{\lambda max}$ ($CHCl_3$) 230; EIMS of TMSi derivative: Figure 2; ^{1}H-NMR: Table 1; ^{13}C-NMR: Table 1.

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