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MONO-TETRAHYDROFURAN ACETOGENINS FROM *GONIOTHALAMUS GIGANTEUS*†

FERAS Q. ALALI, YAN ZHANG, LINGLING ROGERS and JERRY L. MCLAUGHLIN*

Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907, U.S.A.

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Key Word Index—*Goniothalamus giganteus* Annonaceae; brine shrimp; mosquito larvae; acetogenins; goniotetracin; (2,4-*cis* and *trans*)-gonioneninone; ketolactones.

Abstract—Goniotetracin and a mixture of (2,4-*cis* and *trans*)-gonioneninone, new, bioactive, mono-tetrahydrofuran (THF) γ-lactone and ketolactone acetogenins, were isolated from the bark of *Goniothalamus giganteus* (Annonaceae) by activity-directed fractionation using the brine shrimp lethality test (BST). The structures were elucidated based on spectroscopic and chemical methods. Their absolute stereochemistries were determined by the advanced Mosher ester method. Both showed selective and significant cytotoxicities, comparable to the potency of adriamycin, to the human pancreatic tumour cell line (PACA-2), while goniotetracin and goniothalamicin were comparable to the activity of rotenone against yellow fever mosquito larvae.

INTRODUCTION

Goniothalamus giganteus Hook. f. et Thomas (Annonaceae) is a tropical tree widely distributed in southeast Asia. It has been called 'black medicine' and has a great repute as a drug among the Malays [1]. Extracts of the bark, obtained from Thailand, showed toxicities in the brine shrimp test (BST) [2, 3] and showed murine toxicities in the P388 leukemia bioassay [4]. From the ethanol extract of the bark, a number of unusual styryllactones and 20 highly cytotoxic Annonaceous acetogenins have been isolated and reported by our group [5, 6]. The Annonaceous acetogenins are a relatively new class of natural products; the first, uvaricin, was isolated in 1982 [7]; they are C_{35}–C_{37} fatty acid derivatives with a long chain hydrocarbon connected to a variable number of tetrahydrofuran (THF) or tetrahydropyran (THP) rings [8–10].

Through further fractionation work, directed by the BST, we have now isolated two new bioactive acetogenins, goniotetracin (**1**) and a mixture of (2,4-*cis* and *trans*)-gonioneninone (**2**). The structures and absolute stereochemistries were determined by 1-D and 2-D NMR and MS before and after making certain chemical derivatives.

* Author to whom correspondence should be addressed.

† Dedicated to Professor Andre Cavé on the occasion of his retirement from Université Paris-Sud, Châtenay-Malabry, France.

RESULTS AND DISCUSSION

Compound **1** was isolated as a white wax. Its molecular weight was suggested by the mass peak at *m/z* 625 $[MH]^+$ in the CIMS. The HRCIMS gave *m/z* 625.5023 for the $[MH]^+$ ion (calcd 625.5043) corresponding to the molecular formula $C_{37}H_{68}O_7$.

Compound **1** showed an IR carbonyl absorption at 1732 cm^{-1}, an UV (MeOH) λ_{max} at 208 nm ($\log \varepsilon$, 3.52), the proton resonances at δ 7.19, 5.07, 3.83, 2.60, 2.54, 2.50, 2.40, and 1.44, and carbon resonances at δ 174.6, 151.8, 131.2, 77.9, 69.9, and 19.1 all of which provided characteristic spectral features for an α,β-unsaturated γ-lactone fragment with a 4-OH [8, 9].

The presence of four OH resonances in **1** was suggested by a prominent OH absorption at 3418 cm^{-1} in the IR spectrum and was confirmed by four successive losses of H_2O (*m/z* 18) from the $[MH]^+$ in the CIMS and the preparation of the tetra-acetate (**1a**). Compound **1a** gave four singlet protons, at δ 2.04 (10-OAc, 4-OAc) and 2.08 (13-OAc, 18-OAc), and multiplets, at δ 4.84 (H-10, H-13, and H-18) and at δ 5.10 (H-4), corresponding to the downfield shifts of four protons on acetylated secondary OH-bearing carbons. Furthermore, the ^{13}C NMR of **1** showed four carbon resonances due to oxygen-bearing carbons at δ 74.3 (H-18), 74.0 (H-13), 71.6 (H-10), and 69.9 (H-4) indicating the existence of four secondary OH moieties. The presence of a mono-THF ring, with two OH groups flanking the ring, was suggested by proton resonances at δ 3.45 (H-13), 3.82 (H-14, H-17), and

3.41 (H-18), and the carbon peaks at 82.7 (H-17) and 82.6 (H-14); these were directly analogous to similar peaks of other mono-THF acetogenins with two flanking hydroxyls groups, such as goniothalamicin (**3**) [11] and annomontacin [12].

The placement of the mono-THF ring system and the four OH groups of **1** along the aliphatic chain were determined based on the EIMS fragmentation pattern of **1** (Fig. 2). The assignments of the peaks in the ^{1}H NMR spectrum of **1** were based on 1-D ^{1}H and 2-D COSY.

The stereochemistries at C-13/C-14 and C-17/C-18 in **1** were concluded to be *threo*, and the stereochemistry of the THF ring was determined as *trans* by comparison with model compounds of known relative configuration, synthesized by both Harmange *et al.* [13] and Fujimoto *et al.* [14], as well as by comparisons with the reported data for goniothalamicin (**3**) [11] and annomontacin [12]. The absolute configurations at C-18, C-13, C-10, C-4 and C-36 were determined using advanced Mosher ester methodology [15, 16]. The per-(*S*)- and (*R*)-methoxy (fluoromethyl) phenylacetic acid (MPTA) esters (Mosher esters) of **1** were prepared, and COSY ^{1}H NMR analysis allowed the assignment of the absolute configuration at C-18 as *R*; it followed then that positions C-17, C-14, and C-13 were *R* considering their relative stereochemistries. We were unable to assign the absolute stereochemistry of C-10 directly from COSY analysis due to overlapping signals. This was achieved, however, by comparing the (*S*)- and (*R*)-Mosher ester chemical shifts of H-10 in **1** to those of the per-Mosher ester of goniothalamicin (**3**) which is the short chain (C-35) version of **1** [17]. The absolute stereochemistry of **3** has been previously confirmed by preparing the (*S*)- and (*R*)-Mosher ester of the intra-molecular formaldehyde acetal derivative across C-10/C-13 [17, 18]. Using the Hoye models, the absolute stereochemistries at C-4 and C-36 were determined from the Mosher esters as *R* and *S*, respectively [19]. Thus, the structure of **1** was elucidated as illustrated, and it was named goniotetracin.

The mixture of (2,4-*cis* and *trans*)-gonioneninone (**2**) was isolated in the form of an amorphous waxy powder in approximately a 1:1 ratio. The molecular weight of **2** was indicated by a peak at *m/z* 623 for the $[MH]^+$ in the CIMS. The HRCIMS gave *m/z* 623.4861 (calcd 623.4887) for the $[MH]^+$ corresponding to the molecular formula $C_{37}H_{66}O_7$. The IR spectrum showed a strong absorption at 1766 cm^{-1} for a γ-lactone carbonyl and at 1717 cm^{-1} for a ketone carbonyl. Compound **2** was transparent under UV light at 225 nm suggesting that the lactone ring is not α,β-unsaturated. In comparison with (2,4-*cis* and *trans*)-annomontacinone [5] and (2,4-*cis* and *trans*)-goniothalamicinone [20], the ^{1}H NMR and ^{13}C NMR spectra of **2** clearly indicated the presence of a ketolactone moiety; see Tables 2 and 3. In the ^{1}H NMR spectrum of **2**, the resonances at δ 4.39 and 4.55, with combined integrations for one proton, were assigned to H-4 and suggested the presence of the (2,4-*cis* and *trans*)-diastereoisomers at the ketolactone ring moiety, as is typical with these ketolactones [8–10]. In the ^{13}C NMR spectrum, signal pairs at δ 178.3 and 178.8, 43.8 and 44.2, 79.3 and 78.9, and 205.62 and 205.55 were assigned to C-1, C-2, C-4, and C-36, respectively; and they also confirmed the presence of the mixture of (2,4-*cis* and *trans*)-isomers. The NMR assignments of H-2, H-3a, H-3b, H-5a, H-5b, H-35a, and H-35b were based on the analysis of the COSY spectrum of **2**. The presence of an isolated double bond in **2** was determined by the proton signals at δ 5.34 and 5.40 and the carbon signals at δ 128.9 and 130.8. Analyzing the splitting pattern at δ 5.34 and 5.40, *J* coupling values of 11, 7 and 7 were revealed, indicating a *cis* configuration. The position of the double bond was determined at C-21/C-22 from the single-relayed COSY spectrum, which showed correlation cross peaks between H-18 (δ 3.43) and H-20 (δ 2.20).

The remaining part of the structure of **2** exhibited identical ^{1}H and ^{13}C NMR signals for the aliphatic chain bearing a mono-THF ring and three OH groups [8–10]. The existence of the three OH moieties was indicated by an IR hydroxyl absorption at 3415 cm^{-1}, three successive losses of H_2O from the $[MH]^+$ in the CIMS, and the preparation of a tri-acetate (**2a**) derivative. Compound **2a** gave three singlet peaks, at δ 2.04 (10-OAc) and 2.08 (13-OAc, 18-OAc), and a multiplet, at δ 4.83 (H-10, H-13, and H-18). Furthermore, the ^{13}C NMR of **2** showed three resonances due to oxygen-bearing carbons at δ 71.5, 74.3, and 73.5, indicating the existence of three secondary hydroxyls. The almost identical signals of **2** to those of **1** at δ 3.43–3.83 in the ^{1}H NMR spectrum and at δ 71.5–82.6 in the ^{13}C NMR spectrum indicated a similar pattern of a mono-THF flanked with two hydroxyls and another hydroxyl two carbons away from C-13. The carbon skeleton and placement of the ring and three OH groups along the hydrocarbon chain were determined based on the EIMS spectral analysis of **2** (Fig. 3).

The relative stereochemistries at C-13/C-14 and C-17/C-18 of **2** were determined to be *threo*, and the stereochemistry of the THF ring was determined as *trans* by comparing ^{1}H and ^{13}C NMR data to those of the Harmange *et al.* [13] and Fujimoto *et al.* [14] models. The absolute stereochemistries of the carbinol centers of compound **2** were elucidated as 18*R*, 13*R*, and 10*R*, using the advanced Mosher ester methodology [15, 16]. It is worth noting that goniotetracin (**1**), (2,4-*cis* and *trans*)-gonioneninone (**2**), goniothalamicin (**3**) [11], and (2,4-*cis* and *trans*)-goniothalamicinone [20] have identical absolute stereochemistries at C-4, C-10, C-13, C-14, C-17, and C-18; this suggests a common biogenetic origin of these compounds. We, thus, predict that gonionenin (**4**), whose absolute stereochemistry has not been previously determined [22], has the same absolute stereo-

Table 1. NMR spectral data (δ ppm) for **1**, **1b**, and **1c**

	^{13}C NMR	^{1}H NMR (J in Hz)			
proton/carbon	**1**	**1**	**1b**	**1c**	$^{\Delta\delta}$**1b–1c**
1	174.6	—			
2	131.2	—			
3a	33.4	2.40 *dd* (15, 8.5)	2.60 *ddt*	2.67 *ddt*	−0.07
3b	33.4	2.50 *dd*	2.54 *m*	2.58 *m*	−0.04
4	69.9	3.83 *m*	5.30 *m*	5.35 *m*	*R**
5	22.7–37.4	1.45 *m*	1.57, 1.65	1.51, 1.58	+0.06, +0.07
6–9	22.7–37.4	1.18–1.80			
10	71.6	3.64 *m*	4.99 *m*	5.02 *m*	*R**
11–12	22.7–37.4	1.18–1.80			
13	74.0	3.45 *m*	4.94 *m*	4.97 *m*	*R**
14	82.6	3.82 *m*	3.94 *m*	4.01 *m*	−0.07
15e,16e	22.7–37.8	1.69 *m*	1.66, 1.51	1.89, 1.84	−0.23, −0.33
15a,16a	22.7–37.8	2.00 *m*	1.35, 1.24	1.55, 1.47	−0.20, −0.23
17	82.7	3.82 *m*	3.77 *m*	3.94 *m*	−0.17
18	74.3	3.41 *m*	4.87 *m*	4.91 *m*	*R**
19	22.7–37.8	1.41 *m*	1.48 *m*	1.41 *m*	+0.07
20–33	22.7–37.8	1.18–1.80			
34	14.1	0.88 *t* (7.5)	0.88 *t* (7.0)	0.88 *t* (7.5)	
35	151.8	7.19 *q*	6.72 *q*	6.95 *q*	−0.23
36	77.9	5.07 *qq*	4.87 *m*	4.91 *m*	−0.04
37	19.1	1.44 *d* (7)	1.28 *d* (6.5)	1.31 *d* (7.0)	−0.03

* Absolute configuration of carbinol centre.

chemistry as that of (2,4-*cis* and *trans*)-gonioneninone (**2**).

Duret *et al.* have experimentally demonstrated that the ketolactone acetogenins may be derived from the 4-OH-α,β-unsaturated γ-lactone acetogenins through translactonization [21]. It is yet to be proven if this reaction is enzymatic, taking place in the plant cells, or an artifact of the extraction procedures. Since all 4-OH acetogenins found, so far, have the *R* stereochemistry at C-4, the *R* configuration has been assigned for C-4 in **2**. Consequently, the structure of **2** is proposed as illustrated and named (2,4-*cis* and *trans*)-gonioneninone, honoring the parent acetogenin, gonionenin (**4**) [22].

The biological activities of **1–4** are summarized in Table 4. These compounds were all active in the BST [2, 3] and in the yellow fever mosquito larvae mictotiter (YFM) assay [23]; they also showed significant and selective cytotoxicities among the six human tumour cell lines in our seven-day MTT human solid tumour panel [24–28]. Compound **1** was generally more cytotoxic, while **2** appeared to be more selective showing promising activity against the human pancreatic tumour cell line [28]. Compounds **1** and **3** showed potent activities in the yellow fever mosquito assay [23] comparable to that of rotenone.

All of the acetogenins, tested so far, decrease oxygen uptake in mitochondrial tests [29, 30]. These results indicate that they act, at least in part, as potent inhibitors of ATP production via blocking at complex **I** in mitochondria [31]. In addition, they act as potent inhibitors of the plasma membrane NADH oxidase of cancerous cells; this action decreases cytosolic ATP production [32]. The consequence of such ATP deprivation is apoptosis (programmed cell death) [33]. Recently, we have shown that the acetogenins also inhibit cells that are multiple drug resistant (MDR) due to ATP-dependent efflux mechanisms [34, 35], and, thus, they offer a unique mechanism of action for cancer chemotherapy. They are also very effective against pesticide resistant German cockroaches; possibly by the same mechanism [36].

EXPERIMENTAL

Instrumentation

Optical rotations were determined on a Perkin 241 polarimeter. IR spectra (film) were measured on a Perkin-Elmer 1600 FTIR spectrometer. UV spectra were taken in MeOH on a Beckman DU 640 series Spectrophotometer. ^{1}H NMR, ^{1}H–^{1}H COSY, and ^{13}C NMR spectra were obtained on a Varian VXR-500S spectrometer. Low resolution MS data were collected on a Finnigan 4000 spectrometer. High resolution CIMS were performed on a Kratos MS50. HPLC separations were performed with a Rainin Dynamax solvent delivery system (model SD-200) using a Dynamax software system and a silica gel column (Dynamax 60-A 250 × 21 mm) equipped with a Dynamax absorbance detector (model UV-1) set at 225 nm. Analytical TLC was carried out on silica gel plates (0.25

Table 2. ^{1}H NMR spectral data (δ ppm) for **2**, **2b**, and **2c**

	^{1}H NMR (J in Hz)				
proton	**2** *cis*	**2** *trans*	**2b**	**2c**	$^{\Delta\delta}$**2b–2c**
1					
2	3.03 *m*	3.00 *m*			
3a	2.60 *m*	2.23 *m*			
3b	1.48 *m*	2.00 *m*			
4	4.39 *dddd* (11,7.5,5.5,5.3)	4.55 *dddd* (8,8,4.5,4)			
5a,b	1.62, 1.77	1.57, 1.71	1.58, 1.70 *c** —†, 1.66 *t**	1.47, 1.68 *c** 1.49, 1.62 *t**	+0.11, +0.02 —†, +0.04
6–9	1.18–1.80				
10	3.63 *m*		5.00 *m*	5.04 *m*	*R*‡
11–12	1.18–1.80				
13	3.45 *m*		4.96 *m*	4.97 *m*	*R*‡
14	3.83 *m*		3.95 *m*	4.02 *m*	−0.07
15e,16e	1.69 *m*		1.67, 1.54	1.89, 1.84	−0.22, −0.30
15a,16a	2.00 *m*		1.37, 1.27	1.56, 1.47	−0.19, −0.20
17	3.83 *m*		3.78 *m*	3.94 *m*	−0.16
18	3.43 *m*		4.89 *m*	4.92 *m*	*R*‡
19	1.48 *m*		1.40–1.50	1.35–1.55	
20	2.20 *m*		1.98 *m*	1.96 *m*	+0.02
21	5.34 *ddd* (11, 7, 7)		5.25 *m*	5.22 *m*	+0.03
22	5.40 *ddd* (11, 7, 7)		5.40 *m*	5.36 *m*	+0.04
23	2.06 *m*		1.94 *m*	1.89 *m*	+0.05
24–33	1.18–1.80				
34	0.88 *t* (7.5)				
35a	2.60 *dd*	2.67 *dd* (19.5, 9.5)			
35b	3.10 *dd* (18.5, 3.5)	3.03 *dd*			
26	—				
37	2.20 *s*				

* c: *cis*, t: *trans*.
† proton could not be assigned.
‡ Absolute configuration of carbinol centre.

Table 3. ^{13}C NMR spectral data (δ) of **2**

carbon	**2** *cis*	**2** *trans*	carbon	**2** *cis*	**2** *trans*
1	178.3	178.8	19		25.2–37.3
2	43.8	44.2	20		23.3
3a,b		25.2–37.3	21		128.9
4	79.3	78.9	22		130.8
5–9		25.2–37.3	23		27.2
10		71.5	24–31		25.2–37.5
11–12		25.2–37.3	32		31.9
13	74.3	33		22.6	
14		82.6	34		14.1
15–16		25.2–37.3	35a,b		25.2–37.5
17		82.6	36	205.62	205.55
18		73.5	37		36.7

Table 4. Biological data for compounds **1–4**

Compound	BST* LC_{50} (μg/mL)	YFM† LC_{50} (μg/mL)	Cytotoxicity (ED_{50}, μg/mL) A-549‡	MCF-7§	HT-29¶	A-498**	PC-3††	PACA-2‡‡
1	$<2.0\times10^{-1}$	6.0×10^{-1}	3.9×10^{-1}	1.7	1.5	1.5	2.1×10^{-1}	2.6×10^{-2}
2	$<3.0\times10^{-1}$	44	1.8	3.7	2.9	2.2	1.5	4.5×10^{-2}
3	6.0×10^{-1}***	8.0×10^{-1}	2.8×10^{-1}	3.3	2.4	2.4	1.2	1.9
4	22†††	3.3	1.7	5.1	2.3	2.2	1.5	4.5×10^{-1}
rotenone§§	—	2.0×10^{-1}	—	—	—	—	—	—
adriamycin¶¶	—	—	4.5×10^{-3}	9.2×10^{-2}	2.8×10^{-2}	3.2×10^{-2}	3.9×10^{-2}	3.2×10^{-2}

* Brine shrimp lethality test [2, 3]; † Yellow fever mosquito larva test [23]; ‡ Human lung carcinoma [24]; § Human breast carcinoma [25]; ¶ Human colon adenocarcinoma [26]; ** Human kidney carcinoma [24]; †† Human prostate adenocarcinoma [27]; ‡‡ Human pancreatic carcinoma [28]; §§,¶¶ Positive control standard; *** value taken from ref. # 17; ††† value taken from ref. # 22.

mm), developed with $CHCl_3$–MeOH (9:1) and visualized with 5% phosphomolybdic acid in EtOH.

Biossays

The bioactivities of extracts, fractions, and pure compounds were routinely assayed using a test for lethality to brine shrimp larvae (BST) [2, 3]. The yellow fever mosquito larvae mictotiter plate (YFM) assay [23] was used to determine the relative pesticidal activities of compounds **1–4**; rotenone was used as the positive pesticidal control standard. *In vitro* cytotoxicities, against six human tumour cell lines, were carried out at the Purdue Cancer Centre, Cell Culture Laboratory, using standard 7-day MTT assays for A-549 (human lung carcinoma) [24], MCF-7 (human breast carcinoma) [25], HT-29 (human colon adenocarcinoma) [26], A-498 (human kidney carcinoma) [24], PC-3 (human prostate adenocarcinoma) [27] and PACA-2 (human pancreatic carcinoma) [28]. Adriamycin is always used as a positive antitumour control in the same runs.

Plant material

The stem bark of *Goniothalamus giganteus* (B-826538, PR-50604) was collected in Thailand in September 1978 under the auspices of Dr Robert E. Perdue, Medicinal Plant Laboratory, USDA, Beltsville, MD, where voucher specimens are maintained.

Extraction and isolation

The stem bark (10.7 kg) was ground into powder and percolated with 95% ethanol. The dry extract (900 g) (F001) was partitioned between H_2O and CH_2Cl_2 to give a H_2O layer (F002) and a CH_2Cl_2 layer. The residue of the CH_2Cl_2 layer (430 g) (F003) was partitioned between 90% MeOH and hexane, giving a MeOH layer (400 g) (F005) and a hexane layer (30 g) (F006). The MeOH layer (F005) was the most active fraction in the BST (LC_{50} 1.02 μg/ml). Thus, a portion (190 g) of F005 was repeatedly chromatographed over open silica gel columns directed by the BST test [2], using gradients of hexane-acetone, hexane-EtOAc and $CHCl_3$–MeOH, and purified by normal phase HPLC eluted with 10% THF in MeOH–hexane (4–6)% to give the colourless waxy compounds **1** and **2**.

Goniotetracin (**1**). White amorphous powder (4 mg); $[\alpha]_D^{23}$ +16.1° (c 0.056, $CHCl_3$); UV (MeOH) λ_{max} 208 nm ($\log\varepsilon = 3.52$); IR ν_{max} cm^{-1} (film on NaCl plate): 3418, 2919, 2850, 2359, 1732, 1468, 1319, 1206; CIMS (isobutane) *m/z* $[MH]^+$ 625 (32), $[MH-H_2O]^+$ 607 (56), $[MH-2H_2O]^+$ 589 (100), $[MH-3H_2O]^+$ 571 (11), $[MH-4H_2O]^+$ 553 (1.5); EIMS fragments, see (Fig. 2); HRCIMS (isobutane) *m/z* 625.5023 for $C_{37}H_{68}O_7$ (calcd 625.5043); ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1.

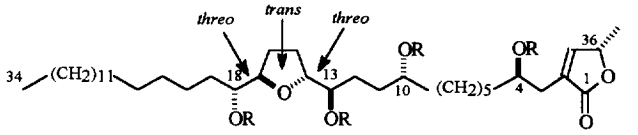
(2,4-cis *and* trans)-*Gonioneninone* (**2**). White amorphous powder (4 mg); $[\alpha]_D^{23}$ +16.0° (c 0.05, $CHCl_3$); IR ν_{max} cm^{-1} (film on NaCl plate): 3415, 2922, 2851, 1766, 1717, 1466; CIMS (isobutane) *m/z* $[MH]^+$ 623 (15), $[MH-H_2O]^+$ 605 (69), $[MH-2H_2O]^+$ 587 (100), $[MH-3H_2O]^+$ 569 (22); EIMS fragments see (Fig. 3); HRCIMS (isobutane) *m/z* 623.4861 for $C_{37}H_{66}O_7$ (calcd. 623.4887); ^1H NMR ($CDCl_3$, 500 MHz), see Table 2; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 3.

Preparation of acetylated derivatives

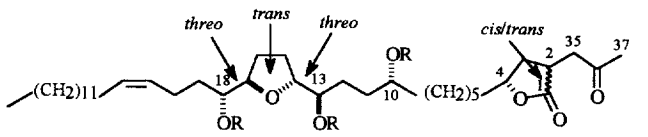
Samples (1–2 mg) of pure acetogenins **1** and **2**, were dissolved in pyridine (1.0 ml), anhydrous Ac_2O (1 ml) was added, and the mixture was set at rt for 4–8 h. The mixture was then partitioned between H_2O and $CHCl_3$, and the organic layer was concentrated and subjected to silica gel microcolumn chromatography to afford the pure actetae derivatives **1a** and **2a**.

Preparation of Mosher esters

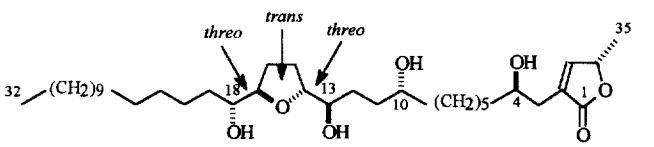
To an acetogenin (0.5–1 mg, in 0.5 ml of CH_2Cl_2) were sequentially added pyridine (0.1 ml), 4-(dime-



1 R=H **1a** R=Ac **1b** R=(*S*)-MPTA **1c** R=(*R*)-MPTA



2 R=H **2a** R=Ac **2b** R=(*S*)-MPTA **2c** R=(*R*)-MPTA



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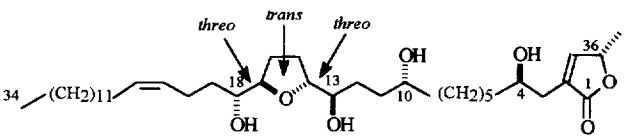

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Fig. 1. Chemical structures of **1-4**

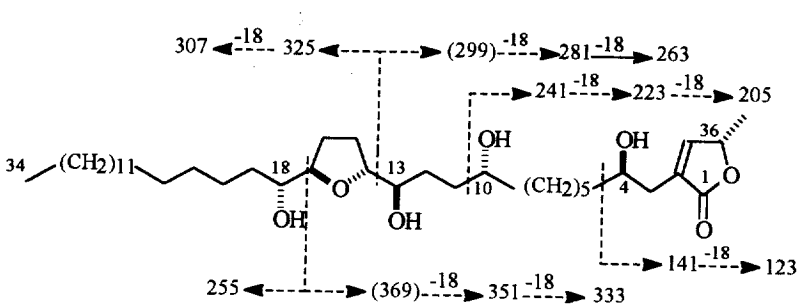

Fig. 2. Diagnostic EIMS fragments of **1**; losses of H_2O indicated by 18 *m/z*

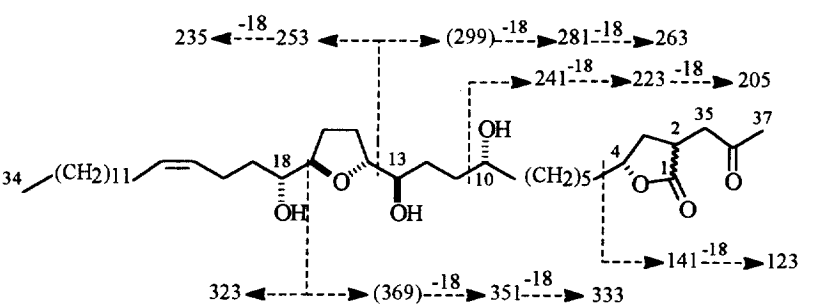

Fig. 3. Diagnostic EIMS fragments of **2**; losses of H_2O indicated by 18 *m/z*.

thylamino)pyridine (0.1 mg), and (*R*)-(−)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride (15 mg). The mixture was stirred at rt from 4 h to overnight, checked with TLC to make sure that the reaction was complete, and passed through a disposable pipet (0.6 × 4 cm) containing silica gel (60–200 mesh), and eluted with 3 ml CH_2Cl_2. The CH_2Cl_2 residue, dried *in vacuo*, was redissolved in 1% $NaHCO_3$ (5 ml) and H_2O (2 × 5 ml); the CH_2Cl_2 layer was dried *in vacuo* to give the (*S*)-Mosher esters. Using (*S*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride gave the (*R*)-Mosher esters. Both yields were typically higher than 90%. For partial ^1H NMR spectroscopic assignments of **1b**, **1c**, **2b**, and **2c**, see Tables 1 and 2.

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