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MONO-THF RING ANNONACEOUS ACETOGENINS FROM *ANNONA SQUAMOSA*

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IN HONOUR OF THE RETIREMENT OF PROFESSOR ANDRÉ CAVÉ

Key Word Index—*Annona squamosa*; brine shrimp assay; Annonaceae; Annonaceous acetogenins; 4-deoxyannoreticuin; *cis*-4-deoxyannoreticuin; (2,4-*cis* and *trans*)-squamoxinone.

Abstract—Continuing work on the bark of *Annona squamosa* Rich. (Annonaceae), directed by the brine shrimp lethality test (BST), has resulted in the isolation of three new Annonaceous acetogenins, 4-deoxyannoreticuin, *cis*-4-deoxyannoreticuin, and (2,4-*cis* and *trans*)-squamoxinone. The first two are additional examples of acetogenins isolated from this plant species which contain the unusual feature of an oxygen functionality at the C-9 position. They have a hydroxylated mono-THF ring with respective *threo*/*trans*/*threo* and *threo*/*cis*/*threo* relative stereochemistries. The latter compound is a ketolactone mixture which has the same relative stereochemistry around the THF ring and the same spatial relationship between the THF ring and the hydroxyl group along the aliphatic chain as 4-deoxyannoreticuin, but is two methylene units longer. Additionally, the isolated hydroxyl group is at C-11, while the THF ring starts at C-17, instead of at C-9 and C-15, respectively, as for the first two compounds. All three compounds showed moderate, but significant, cytotoxicities against a panel of six human tumor cell lines with (2,4 *cis* and *trans*)-squamoxinone showing promising selectivity against the pancreatic cell line (PACA-2).

INTRODUCTION

The Annonaceous acetogenins are an expanding class of potent long chain fatty acid derivatives which are found only in species of the Annonaceae. Interest in these compounds has become worldwide as knowledge of their remarkable antitumor and pesticidal activities has spread [1, 2]. The total number of Annonaceous acetogenins now exceeds 240 [1, 3]. *Annona squamosa* Rich. (Annonaceae), commonly referred to as the custard apple, a fruit tree native to Central America, is cultivated throughout the tropics [4]. Prior work by Fujimoto's group led to the isolation of 26 different acetogenins from the seeds of this species [5]. Our previous work on the bark extract resulted in the isolation of three new and eight known acetogenins [6, 7].

Through further fractionation work, directed by the brine shrimp lethality test (BST) [8], we have now isolated three new bioactive acetogenins, 4-deoxyannoreticuin (**1**), *cis*-4-deoxyannoreticuin (**2**), and (2,4-*cis* and *trans*)-squamoxinone (**3**). Compounds **1** and **2** are mono-THF ring compounds with three hydroxyl groups, whereas **3** is a mixture of ketolactone acetogenins which also have one THF ring and three hydroxyl groups.

RESULTS AND DISCUSSION

4-Deoxyannoreticuin (**1**), a white amorphous powder, is similar to annoreticuin [9] except that it does not have a hydroxyl group at C-4 (Fig. 1). The MH^+ peak in the FABMS at *m/z* 581 established the molecular weight as 580. The elemental composition of $C_{35}H_{64}O_6$ was confirmed by high resolution CIMS of the molecular ion which gave an exact mass of *m/z* 581.4763 (calcd 581.4781). Diagnostic peaks in the ^1H NMR spectrum at δ 6.99 (H-33), 5.00 (H-34), and 1.41 (H-35) (Table 1), and ^{13}C NMR signals at δ 174.02 (C-1), 134.45 (C-2), 148.94 (C-33), 77.44 (C-34), and 19.20 (C-35) (Table 2) indicated the presence of an α,β-unsaturated γ-lactone. This was corroborated by an absorption band in the IR of **1** at 1760 cm^{-1} and the UV λ_{max} at 215 nm. A multiplet in the ^1H NMR spectrum at δ 2.27 (H-3), as well as the other, slightly upfield-shifted, lactone signals, demonstrated that this compound did not have a hydroxyl

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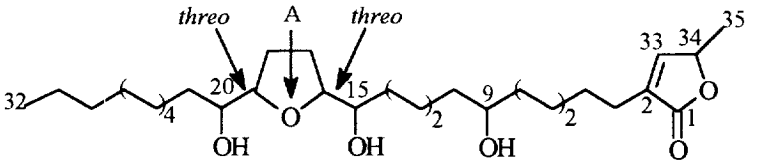

1: A = *trans*
2: A = *cis*

Fig. 1.

Table 1. ^{1}H NMR spectral data (δ) for **1–3**

	^{1}H NMR (500 MHz, $CDCl_3$, *J* in Hz)			
Position	**1**	**2**	**3** *trans*	**3** *cis*
1	—	—	—	—
2	—	—	3.02 *m*	3.03 *m*
3a	2.27 *m*	2.27 *m*	2.23 *ddd* (12.9, 9.6, 3.4)	2.60 *ddd* (12.3, 9.4, 5.6)
3b	2.27 *m*	2.27 *m*	1.99 *m*	1.48 *m*
4	1.57 *m*	1.57 *m*	4.56 *dddd* (8.3, 8.2, 5.7, 3.2)	4.40 *dddd* (10.7, 7.4, 5.4, 5.4)
5a, 5b	1.26 *br s*	1.26 *br s*	1.70 *m*, 1.57 *m*	1.75 *m*, 1.62 *m*
6–7	1.26 *br s*	1.26 *br s*	1.26 *br s*	1.26 *br s*
8	1.41 *m*	1.41 *m*	1.26 *br s*	1.26 *br s*
9	3.58 *m*	3.58 *m*	1.26 *br s*	1.26 *br s*
10	1.41 *m*	1.41 *m*	1.42 *m*	1.42 *m*
11	1.26 *br s*	1.26 *br s*	3.59 *m*	3.59 *m*
12	1.26 *br s*	1.26 *br s*	1.42 *m*	1.42 *m*
13	1.26 *br s*	1.26 *br s*	1.26 *br s*	1.26 *br s*
14	1.41 *m*	1.41 *m*	1.26 *br s*	1.26 *br s*
15	3.41 *m*	3.42 *m*	1.26 *br s*	1.26 *br s*
16	3.80 *m*	3.82 *m*	1.41 *m*	1.41 *m*
17	1.99 *m*, 1.68 *m*	1.94 *m*, 1.75 *m*	3.41 *m*	3.41 *m*
18	1.99 *m*, 1.68 *m*	1.94 *m*, 1.75 *m*	3.80 *m*	3.80 *m*
19	3.80 *m*	3.82 *m*	1.99 *m*, 1.69 *m*	1.99 *m*, 1.69 *m*
20	3.41 *m*	3.42 *m*	1.99 *m*, 1.69 *m*	1.99 *m*, 1.69 *m*
21	1.41 *m*	1.41 *m*	3.80 *m*	3.80 *m*
22	1.26 *br s*	1.26 *br s*	3.41 *m*	3.41 *m*
23	1.26 *br s*	1.26 *br s*	1.41 *m*	1.41 *m*
24–30	1.26 *br s*	1.26 *br s*	1.26 *br s*	1.26 *br s*
31	1.28 *m*	1.28 *m*	1.26 *br s*	1.26 *br s*
32	0.88 *t* (7.0)	0.88 *t* (7.0)	1.26 *br s*	1.26 *br s*
33	6.99 *m*	6.99 *m*	1.26 *br s*	1.26 *br s*
34	5.00 *dq* (1.5, 7.0)	5.00 *dq* (1.5, 7.0)	0.88 *t* (7.0)	0.88 *t* (7.0)
35a	1.41 *d* (6.5)	1.41 *d* (6.5)	2.66 *dd* (19.5, 9.0)	2.61 *dd* (18.5, 9.0)
35b	1.41 *d* (6.5)	1.41 *d* (6.5)	3.05 *dd* (19.5, 3.0)	3.11 *dd* (18.5, 3.0)
36	—	—	—	—
37	—	—	2.20 *s*	2.20 *s*

at the C-4 position, a common feature in many acetogenins [1, 10]. Three successive losses of water from the MH^+ ion in the CIMS suggested that the molecule contained three hydroxyls. This was further supported by a broad absorbance in the IR spectrum at 3400 cm^{-1}. The presence of a single THF ring with two flanking hydroxyls in a *threo/trans/threo* relative configuration was evidenced by ^{1}H NMR signals at δ 3.41 (H-15/20) and 3.80 (H-16/19), each integrating for two protons, as well as ^{13}C NMR peaks at δ 74.06 (C-

Table 2. ^{13}C NMR spectral data (δ) for **1**–**3**

Position	^{13}C NMR (125 MHz, $CDCl_3$)			
	1	**2**	**3** *trans*	**3** *cis*
1	174.02	174.54	178.31	178.86
2	134.45	134.73	43.78	44.24
3	25.13*	25.10*	34.40*	34.40*
4	28.72–29.70	29.20–29.64	78.87	79.32
5	28.72–29.70	29.20–29.64	36.67*	36.67*
6	28.72–29.70	29.20–29.64	25.57†	25.57†
7	25.13*	25.29*	29.20–29.96	29.20–29.96
8	37.40†	37.27†	29.20–29.96	29.20–29.96
9	71.90	71.86	25.52†	25.52†
10	37.40†	37.48†	37.25*	37.25*
11	25.59*	25.50*	71.86	71.86
12	28.72–29.70	29.20–29.64	37.46*	37.46*
13	25.59*	25.58*	25.46†	25.46†
14	33.42†	34.13†	29.20–29.96	29.20–29.96
15	74.06	74.37	25.20†	25.20†
16	82.58‡	82.67	33.47*	33.47*
17	28.72	28.12	74.02‡	74.02‡
18	28.72	28.12	82.63§	82.63§
19	82.64‡	82.67	28.70	28.70
20	74.06	74.37	28.70	28.70
21	33.42†	34.13†	82.58§	82.58§
22	25.59*	25.69*	73.97‡	73.97‡
23	28.72–29.70	29.20–29.64	33.25*	33.25*
24	28.72–29.70	29.20–29.64	25.12†	25.12†
25–29/31	28.72–29.70	29.20–29.64	29.20–29.96	29.20–29.96
30/32	31.91†	31.90†	31.88*	31.88*
31/33	22.68	22.66	22.65	22.65
32/34	14.11	14.08	14.08	14.08
33/35	148.94	148.94	35.40*	35.32*
34/36	77.44	77.40	205.61	205.61
35/37	19.20	19.19	25.12*	25.12*

*†‡§ Values may be interchangeable in each column.

15/20), 82.58 (C-16/C-19), and 82.64 (C-16/C-19) [5, 10]. The ring was placed between C-15 and C-20 based on peaks in the EIMS at *m/z* 311 and 381. A third hydroxyl attached somewhere along the aliphatic chain could be identified by a multiplet at δ 3.58 in the ^1H NMR and a signal at δ 71.90 in the ^{13}C NMR spectra [10]. The peak in the EIMS at *m/z* 311 indicated that this hydroxyl was between the THF and lactone rings. A small EIMS peak at *m/z* 211 suggested that the location may be at C-9. A high resolution EIMS of the peak at *m/z* 211 gave an exact mass of *m/z* 211.1342 (calcd 211.1334), established the molecular composition of the fragment as $C_{12}H_{19}O_3$, and confirmed that the hydroxylation was, indeed, at C-9. Unfortunately, insufficient material was available to complete Mosher ester derivatizations [11–13]. Therefore, **1** is presented here in its two-dimensional form, and the structure was concluded to be as illustrated and named 4-deoxyannoreticuin. This compound, along with **2**, continues the series of acetogenins isolated from this plant which share the unusual characteristic of an oxygen functionality, either a carbonyl or hydroxyl group, at C-9 [6, 7].

The spectral data showed that **2** is similar to **1** in that it has one THF ring and two flanking hydroxyls with an additional hydroxyl group at C-9. The only difference is in the relative stereochemistry of the THF ring which is *threo/cis/threo* for **2**. Compound **2** was also isolated as a white amorphous powder. The MH^+ peak at *m/z* 581 in the FABMS of **2** established the mass as 580 daltons. High resolution CIMS of the molecular ion gave an exact mass of *m/z* 581.4763 (calcd 581.4781) which confirmed the molecular formula to be $C_{35}H_{64}O_6$, the same as **1**. The presence of an α,β-unsaturated γ-lactone in the structure of **2** was determined by signals in the ^1H NMR (Table 1), ^{13}C NMR (Table 2), UV and IR spectra which were very similar to those for **1**. Once again the presence of three hydroxyl groups was indicated by a broad absorbance in the IR spectrum at 3426 cm^{-1} and three successive losses of water from the MH^+ ion in the CIMS. As in **1**, a single THF ring with two flanking hydroxyls was

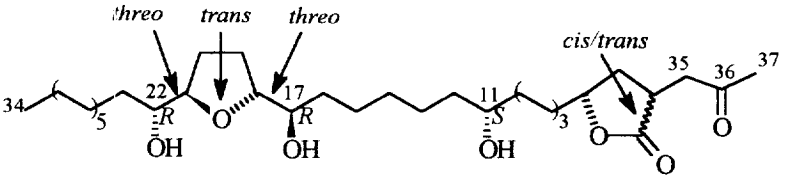

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Fig. 2.

evidenced by ^{1}H NMR signals at δ 3.42 (H-15/20) and 3.82 (H-16/19). NMR signals for protons for the ring methylenes at δ 1.94 and 1.75 (H-17/18) and carbon-13 at δ 74.37 (C-15/20), 82.67 (C-16/19), and 28.12 (C-17/18) matched those for other acetogenins with a *cis* ring and were in agreement with synthetic models produced by Fujimoto's group, thus establishing the relative stereochemistry around the THF ring as *threo/cis/threo* [5, 14–16]. The position of the ring along the aliphatic chain was established again by fragments in the EIMS at *m/z* 311 and 381. As before, the presence of an additional hydroxyl group was indicated by a ^{1}H NMR multiplet at δ 3.58 and by a ^{13}C NMR peak at δ 71.86. The position of the hydroxyl in **2** was also determined to be at C-9 by high resolution EIMS of the fragment ion at *m/z* 211.1340 (calcd 211.1334). Again, the limited amount of isolate precluded Mosher ester preparations, and only the planar structure can be presented. Therefore, the structure of **2** was determined as illustrated and named *cis*-4-deoxy annoreticuin.

Compound **3** was isolated in a mixture as a white amorphous powder. The MH^+ ion at *m/z* 625 in the FABMS of this compound established the molecular mass at 624. The molecular formula of $C_{37}H_{68}O_7$ was proven by the high resolution CIMS peak for the molecular ion at *m/z* 625.5062 (calcd 625.5043). The presence again of a broad IR absorption band at 3445 cm^{-1} coupled with the loss of three successive molecules of water from the MH^+ ion in the FABMS suggested the existence of three OH groups in the structure of **3**. Unlike the first two compounds, **3** is a mixture of *cis*- and *trans*- ketolactone isomers and, as is usual for these mixtures, they were not separated in the isolation procedure [17, 18]. This arrangement of the lactone was evidenced by the low UV λ_{max} at 203 nm. The disappearance of ^{1}H NMR signals at δ 7.18 and 5.06 and the appearance of peaks at δ 4.40 and 4.54 (H-4), 2.20 (H-37), and between δ 3.11 and 2.00 (H-2, 3, and 35) (Table 1) also suggested the *cis/trans* ketolactone mixture. Pairs of ^{13}C NMR resonances at δ 178.31 and 178.86 (C-1), 43.78 and 44.24 (C-2), 78.87 and 79.32 (C-4) and 205.61 (C-36) (Table 2) further substantiated this assignment. Like **1** and **2**, compound **3** has one THF ring with two flanking hydroxyls and an additional hydroxyl group six methylene units away. However, the ring and flanking hydroxyls in **3** begin at C-17, and the additional hydroxyl group is at C-11 instead of at C-15 and C-9 as in **1** and **2**; in addition, **3** is 37 carbons long instead of 35 as in **1** and **2**. These structural features for **3** were established based on EIMS peaks at *m/z* 355 for cleavage next to the THF ring and at *m/z* 255 for fragmentation next to the C-11 hydroxyl. High resolution EIMS of the fragment at *m/z* 255 established its exact mass as 255.1590 which corresponds to a molecular composition of $C_{14}H_{23}O_4$ (calcd 255.1596) and confirmed the location of the hydroxyl at C-11. The relative stereochemistry across the THF ring was determined to be *threo/trans/threo* from ^{1}H NMR signals at δ 3.41 (H-17/22) and 3.80 (H-18/21) and ^{13}C NMR signals at δ 74.02 (C-17), 73.97 (C-22), 82.63 (C-18), and 82.58 (C-21). The hydroxyl at C-11 appeared as a multiplet at δ 3.59 in the ^{1}H NMR and at δ 71.86 in the ^{13}C NMR spectrum.

The absolute stereochemistries of the chiral centers in **3** were determined by preparing the tri-*S* and tri-*R* Mosher ester derivatives (**3a** and **3b**, respectively). Analysis of the ^{1}H-^{1}H COSY for **3a** and **3b** demonstrated that the absolute stereochemistries at C-15 and C-20 were both *R* (Table 3). For C-11, assignment of the absolute stereochemistry as *S* was made based on small but consistent differences between **3a** and **3b** in the chemical shifts of the protons at H-3, H-4, and H-5. The stereochemistry at C-4 was assumed as *R* based on spectral comparisons with (2,4-*cis* and *trans*)-bullatacinone, which has known chirality [17], and the fact that all 4-oxygenated acetogenins found to date are 4*R*. Thus, the structure of **3** was elucidated as illustrated and named (2,4-*cis* and *trans*)- squamoxinone.

Compounds **1**–**3** all showed moderate but significant cytotoxicities in a panel of six human tumor cell lines (Table 4). Compound **3**, as with a series of 9-carbonyl acetogenins previously reported from this species [6], showed promising selectivity for the pancreatic cell line (PACA-2). Past structure activity relationship studies have revealed that mono-THF ring compounds are generally the least active of the acetogenins, particularly when they lack a hydroxyl at the four position [19–22]. Acetogenins containing two adjacent THF rings have been found to be the most potent members of this group followed by compounds possessing two non-adjacent THF rings. The acetogenins act, in part, by blocking the flow of electrons through complex I, and by inhibiting the NADH

Table 3. ^{1}H NMR (500 MHz, $CDCl_3$) data (δ) for MTPA derivatives of **3**

MTPA ester	5 *cis*	5 *trans*	16	18	19	20	21	23
1a	1.71, 1.51	1.74, 1.59	1.57	3.92	1.65	1.39	3.92	1.57
1b	1.67, 1.53	1.69, 1.55	1.53	4.00	1.92	1.56	4.00	1.53
$\Delta(\delta S - \delta R)$	neg.	neg.	pos.	neg.	neg.	neg.	neg.	pos.
Configuration	11*S*		17*R*			22*R*		

Table 4. Biological data for compounds **1–3**

		Cytotoxicity (ED_{50}, μg/ml)					
Compound	(LC_{50}, μg/ml)	A-549†	MCF-7‡	HT-29§	A-498¶	PC-3	PACA-2**
1	8.93	3.87	2.23	1.69	2.23	2.66	2.88
2	6.75	1.99	1.74	1.42	1.84	2.08	1.09
3	2.70	1.89	1.71	1.44	1.48	2.22	0.0045
Adriamycin††	0.26	0.039	0.13	0.37	0.033	0.24	0.025

* Brine shrimp lethality test [8].
† Human lung carcinoma [27].
‡ Human breast adenocarcinoma [28].
§ Human colon adenocarcinoma [29].
¶ Human kidney carcinoma [28].
‖ Human prostate adenocarcinoma [30].
** Human pancreatic carcinoma [31]
†† Positive control, BST LC_{50} value taken from Ref. 32.

oxidase enzyme peculiar to the plasma membranes of cancerous cells [23, 24]; both actions cause depletion of ATP; as a result, they show special promise against multi-drug resistant tumors and insecticide resistant insects which may have ATP-dependent efflux pumps [25, 26].

EXPERIMENTAL

Instrumentation

UV spectra were measured on a Beckman DU 640 series spectrophotometer. IR data were collected using a Perkin–Elmer 1600 series FTIR instrument. Optical rotations were obtained on a Perkin–Elmer model 241 polarimeter. ^{1}H NMR and ^{13}C NMR were run on a Varian VXR-500S spectrometer. Low-resolution EIMS and CIMS data were collected on a Finnigan 4000 spectrometer. High-resolution EIMS, CIMS, and FABMS were obtained on the Kratos MS50 through peak matching. HPLC was carried out using a Dynamax UV-1 detector coupled with a Rainin model HPXL solvent delivery system for normal phase and Dynamax model SDS-200 solvent delivery system for reversed-phase.

Plant material

The dried stem bark of *Annona squamosa* Rich. was purchased from United Chemical and Allied Products in Calcutta, India.

Extraction and isolation

The dried and pulverized bark (7.4 kg) was extracted with EtOH (1.83 kg *F*001, BST LC_{50} = 1.55 ppm). The residue was partitioned between CH_2Cl_2 and H_2O to yield a CH_2Cl_2 soluble residue (842 g *F*003, BST LC_{50} = 1.68 ppm) and a H_2O soluble residue (128.6 g *F*002, BST LC_{50} = 950.14 ppm). *F*003 was further partitioned between 90% aq. MeOH and hexane resulting in a MeOH soluble residue (545.5 g *F*005, BST LC_{50} = 1.52 ppm) and a hexane soluble residue (162.9 g *F*006, BST LC_{50} = 122.97 ppm). A sample (500.5 g) of *F*005 was separated by column chromatography over Si gel using mixtures of hexane and $CHCl_3$ then mixtures of $CHCl_3$ and MeOH as solvent systems. Sixty fractions were collected, and fractions 30–36 (21.67 g) were combined on the basis of TLC and further resolved on another Si gel column eluted with mixtures of hexane and Me_2CO. The pools from this column which were bioactive in the BST were subjected to a third Si gel column eluted with mixtures of $CHCl_3$ and MeOH. Compounds **1–3** were purified by repeated normal-phase and reversed-phase HPLC using solvent systems of hexane:MeOH:THF (90:9:1) and acetonitrile:H_2O (70:30), respectively.

Biological testing

The cytotoxicity of column fractions and pure compounds was monitored using the brine shrimp lethality

test [8]. Cell culture assays were performed in the Purdue Cell Culture Laboratory, Purdue Cancer Center, using standard protocols in 7-day MTT assays with adriamycin as the positive control.

4-*Deoxyannoreticuin* (**1**). White amorphous powder (1.9 mg, 2.6×10^{-5}% yld.); $[\alpha]_D^{23}+6.8^{\circ}$ (c 0.029; CH_2Cl_2); UV (MeOH) λ_{max} 215 (log ε 3.50); IR ν_{max} film cm^{-1}: 3400, 2920, 2853, 1760; FABMS *m/z* $[MH]^+$ 581 (80), $[MH\text{-}H_2O]^+$ 563 (25), $[MH\text{-}2H_2O]^+$ 545 (30), $[MH\text{-}3H_2O]^+$ 527 (100); EIMS *m/z* 345 (14), 311 (4), 293 (40), 275 (27), 211 (7), 193 (4); HRCIMS (isobutane) *m/z* 581.3763 for $C_{35}H_{64}O_6$ (calcd 581.4781); HREIMS *m/z* 211.1342 for $C_{12}H_{19}O_3$ (calcd 211.1334); ^1H NMR spectra data ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR data ($CDCl_3$, 125 MHz), see Table 2.

Cis-4-*deoxyannoreticuin* (**2**). White amorphous powder (2.5 mg, 3.4×10^{-5}% yld.); $[\alpha]_D^{23}+6.8^{\circ}$; (c 0.015, CH_2Cl_2); UV (MeOH) λ_{max} 218 nm (log ε 3.61); IR ν_{max} film cm^{-1}: 3423, 2926, 2854, 1755; CIMS (isobutane) *m/z* $[MH]^+$ 581 (100), $[MH\text{-}H_2O]^+$ 563 (38), $[MH\text{-}2H_2O]^+$ 545 (16), $[MH\text{-}3H_2O]^+$ 527 (8); EIMS *m/z* 345 (12), 311 (4), 293 (100), 275 (44), 211 (6), 193 (5); HRCIMS (isobutane) *m/z* 581.4763 for $C_{35}H_{64}O_6$ (calcd 581.4781); HREIMS *m/z* 211.1342 for $C_{12}H_{19}O_3$ (calcd 211.1334); ^1H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2.

(2,4-*cis and trans*)-*Squamoxinone* (**3**). White amorphous powder (3.6 mg, 4.9×10^{-5}% yld.); $[\alpha]_D^{23}+13.3^{\circ}$ (c 0.023, CH_2Cl_2); UV (MeOH) λ_{max} 203 nm (log ε 3.20); IR ν_{max} film cm^{-1}: 3445, 2919, 2850, 1755; FABMS *m/z* $[MH]^+$ 625 (100), $[MH\text{-}H_2O]^+$ 607 (9), $[MH\text{-}2H_2O]^+$ 599 (22), $[MH\text{-}3H_2O]^+$ 571 (24), $[MH\text{-}4H_2O]^+$ 553 (19); EIMS *m/z* 407 (1), 389 (2), 371 (1), 337 (6), 319 (6), 255 (3); HRCIMS *m/z* 625.5062 for $C_{37}H_{68}O_7$ (calcd 625.5043); HREIMS *m/z* 255.1590 for $C_{14}H_{23}O_4$ (calcd 255.1596); ^1H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2.

Mosher ester derivatization

To **3** (0.5 mg in 0.5 ml CH_2Cl_2) were sequentially added pyridine (0.2 ml), 4-(dimethylamino)-pyridine (0.5 mg), and (*R*)-(−)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride or (*S*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride (12 mg). After 4 hr at room temp., the mixture was passed through a small pipet column (0.6×6 cm) packed with Si gel and eluted with CH_2Cl_2 (5 ml). This residue was then washed with 1% $NaHCO_3$ (5 ml) and H_2O (2×5 ml). The organic layer was evaporated to give the *S*-Mosher ester of **3**. Use of (*S*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride yielded the *R*-Mosher ester of **3**.

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