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ARATICULIN, A *BIS*-TETRAHYDROFURAN POLYKETIDE FROM *ANNONA CRASSIFLORA* SEEDS

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Key Word Index—*Annona crassiflora*; Annonaceae; araticum; polyketide; araticulin.

Abstract—A new tetrahydroxy adjacent bis-tetrahydrofuran polyketide, araticulin, has been isolated from the cytotoxic ethanolic extract of *Annona crassiflora* seeds. Its structure was established by NMR and mass spectrometry as a *threo-trans-threo-trans-threo* isomer of purpureacin-2, the only previously known bis-tetrahydrofuran polyketide bearing an OH group at C-12.

INTRODUCTION

Annona crassiflora, known popularly as 'araticum', is a native Brazilian tree found in the 'cerrado' area. Its fruit is edible and the seeds are traditionally used in folk medicine against snake bites [1]. A number of Annonaceae species of the genus *Annona* have been shown to contain polyketides with significant cytotoxic, antitumour, pesticidal, antimicrobial and antiparasitic activities [2, 3]. We have recently described a cytotoxic nonadjacent bis-tetrahydrofuran polyketide from the petrol extract of *A. crassiflora* seeds that we named crassiflorin [4]; this was shown to be identical to cherimolin-2 or bullatanocin [5, 6].

In the present paper, we report the structural determination of a new polyketide, named araticulin (**1**), which was isolated from the cytotoxic ethanolic extract of *A. crassiflora* seeds.

RESULTS AND DISCUSSION

A 75% ethanol extract of *A. crassiflora* seeds exhibited significant *in vitro* cytotoxicity to human lung carcinoma (A-549) and melanoma (RPMI 7951) cells.

Compound **1** was isolated by a combination of open column chromatography on silica gel and washing with pH 4 buffer. It was obtained as a whitish wax, mp 65–67°. The FAB mass spectrum showed an $[M + H]^+$ at m/z 639.51, corresponding to the molecular formula $C_{37}H_{66}O_8$. Spectral characteristics of **1** and its derivatives, including IR, ^1H NMR, ^{13}C NMR, HREI and FAB mass spectrometry, suggested that it belonged to the rare class of adjacent bis-tetrahydrofuran (THF) polyketides [2, 3].

On the basis of its spectral data (Table 1), **1** was suggested to be a new polyketide possessing a terminal α,β-unsaturated γ-lactone with a 4-OH group [2, 3].

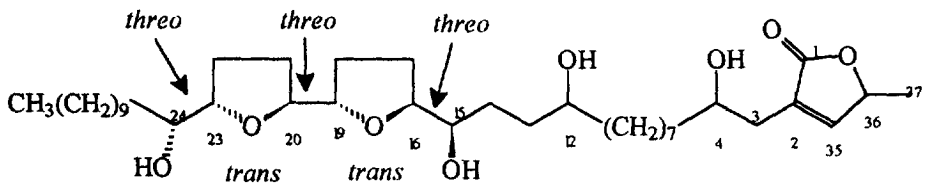

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Table 1. ^{13}C NMR and ^{1}H NMR data for compound **1** and its tetra-acetate ($CDCl_3$)

C-Atom*	**1** (50 MHz)	H-Atom*	**1** (200 MHz)	Araticulin tetra-acetate (500 MHz)
1	174.63	3a	2.51 *dd*	2.52
2	131.17	3b	2.37 *dd*	—
3	33.40	4	3.84 *m*	5.11 *m*
4	69.96	5	1.54 *m*	1.67
5	37.33	11	1.63 *m*	1.67
6–10	29.3–29.7	12	3.59 *m*	4.58 *m*
11	37.5	13–14	1.63 *m*	—
12	71.67	15	3.39 *m*	4.85 *m*
13	33.53	16	3.84 *m*	3.98 *m*
14	31.89	17–18	1.67–1.96 *m*	1.97 *m*
15	74.04†	19	3.84 *m*	3.90 *m*
16	83.17‡	20	3.84 *m*	3.90 *m*
17–18	28.37–28.96	21–22	1.67–1.96 *m*	1.97 *m*
19	81.74	23	3.84 *m*	3.98 *m*
20	81.85	24	3.39 *m*	4.85 *m*
21–22	25.64–25.47	34	0.86 *t*	0.87 *t*
23	82.84‡	35	7.17 *d*	7.07 *s*
24	74.15†	36	5.05 *ddd*	5.09 *ddd*
25	33.32	37	1.41 *d*	1.39 *d*
26–33	28.37–28.96	4 OAc	—	2.022
34	14.10	12 OAc	—	2.034
35	151.79	15 OAc	—	2.074
36	77.97	24 OAc	—	2.070
37	19.10	—	—	—

*Biogenetic numbering.

†,‡Assignments in vertical column interchangeable.

The adjacent bis-THF ring system, with the usual OH groups on each side, was indicated by the ^{13}C NMR ($CDCl_3$) signals of **1** (Table 1) at δ 74.15, 83.17, 82.84, 81.85, 81.74 and 74.04, and ^{1}H NMR chemical shifts (Table 1) at δ 3.39 (H-15, H-24) and 3.84 (*m*, H-16, H-19, H-20 and H-23). The signals in the ^{1}H and ^{13}C NMR spectra of **1** at δ 3.59 and 71.67 are characteristic of a hydroxyl group in an alkyl chain [5].

The THF rings were located from C-16 to C-20 in the hydrocarbon chain on the basis of typical fragments observed in the EI mass spectrum of **1** and its trimethylsilyl derivative. Allocation of the fourth hydroxyl group at C-12 was based on the occurrence of a fragment ion at *m/z* 269.1750 ($C_{15}H_{25}O_4$, calc. 269.17522) due to the cleavage between C-12 and C-13; this was confirmed by the EI mass spectrum of the corresponding trimethylsilyl derivative by the fragment ion at *m/z* 413.3.

The relative stereochemistry around the two THF rings was determined with the aid of empirical ^{1}H and ^{13}C NMR shift rules established with a series of model compounds [7, 8]. Comparison of ^{1}H NMR data for the tetra-acetyl derivative (Table 1) and model compounds [7], indicated that the chemical shifts at δ 4.85 (H-15, H-24), 3.98 (H-16, H-23), 3.90 (H-19, H-20) and 2.07 (15-OAc, 24-OAc) shown by araticulin tetra-acetate were close to those for the *threo-trans-threo-trans-threo* model, suggesting this relative stereochemistry for the THF moieties (C-15/C-16, C-16/C-19, C-19/C-20, C-20/C-23 and C-23/C-24).

The structure of **1** is closely related to that of purpureacin-2, isolated from *A. purpurea* leaves [9]. These two isomeric compounds are the first representatives of adjacent bis-THF Annonaceous polyketides bearing a hydroxyl group at C-12. However, the minor differences in the ^{13}C and ^{1}H NMR chemical shifts suggest that **1** is a new stereoisomer of purpureacin-2 whose relative stereochemistry was not reported by Cepleanu *et al.* [9]. However, the stereochemistry at C-4, C-12 and C-36 in **1** are unknown.

Compound **1** showed significant activities against human tumour cell lines in culture when compared with adriamycin (Table 2).

EXPERIMENTAL

General. Mps: uncorr. IR: KBr. ^{13}C NMR: 50 MHz, $CDCl_3$. ^{1}H NMR: 200 and 500 MHz, $CDCl_3$.

Plant material. Fruits of *A. crassiflora* were collected in Itatiaiuçu and Curvelo, Minas Gerais, Brazil. Plant material was identified by J. L. Pedersoli, Instituto de Ciências Biológicas, UFMG, Belo Horizonte, Minas Gerais, Brazil. A voucher specimen is deposited at the BHCB, UFMG, Belo Horizonte.

Bioassays. Crude extracts were evaluated for cytotoxicity at the Ohio State University, using standard protocols for A 459 (human lung carcinoma), HT-29 (human colon adenocarcinoma), MCF-7 (human breast carcinoma), RPMI 7951 (melanoma) and U 251 (CNS carcinoma).

Table 2. Bioactivities of **1** on human tumor cell lines

Compound	ED_{50} ($\mu g\ ml^{-1}$)				
	A-549*	HT-29†	MCF-7‡	RPMI-7951§	U-251‖
Araticulin (**1**)	6×10^{-3}	6×10^{-1}	4×10^{-3}	10^{-3}	10^{-2}
Adriamycin¶	4×10^{-3}	3×10^{-3}	2×10^{-3}	5×10^{-3}	1×10^{-2}

*Human lung carcinoma.
†Human colon adenocarcinoma.
‡Human breast carcinoma.
§Melanoma.
‖Human CNS carcinoma.
¶Positive control standard.

Extraction and isolation. Powdered seeds (1.25 kg) were successively extracted in a Soxhlet apparatus with petrol and 75% EtOH. The EtOH extract (91.1 g) was fractionated on a silica gel column with a gradient of hexane–CH_2Cl_2–EtOAc–MeOH to give 82 frs. A CH_2Cl_2 soln of the residue (1.4 g) of the fr. eluted with EtOAc was washed with a buffer soln at pH 4. The residue from the organic layer gave a mixt. of a yellow wax and a white powder. The wax was extracted with EtOAc and the residue recrystallized from EtOAc to give a white amorphous powder (40 mg) of **1**.

Araticulin (**1**). $C_{37}H_{66}O_8$. $[\alpha]_D$ +9.6° (MeOH; *c* 0.27). IR ν_{max} cm^{-1}: 3450, 2920, 2840, 1750, 1600, 1450, 1300, 1060, 1020, 920. FABMS (3-NBA), *m/z*: 639.51 $[MH]^+$, 621.56 $[MH - H_2O]^+$, 603.59 $[MH - 2H_2O]^+$, 585.59 $[MH - 3H_2O]^+$, 567.59 $[MH - 4H_2O]^+$. EI-MS: 467, 449, 397, 379, 369, 309, 241, 269, 171, 141. EI-MS, TMSi derivative *m/z*: 683, 613, 593, 543, 453, 413, 383, 313, 243, 213. ^{13}C NMR (50 MHz, $CDCl_3$). Table 1. ^1H NMR (200 and 500 MHz, $CDCl_3$), Table 1.

Araticulin tetraacetate. FABMS (*m*-NBA), *m/z* 807.79 $[MH]^+$, 747.69 $[MH - AcOH]^+$, 687.54 $[MH - 2AcOH]^+$, 627.56 $[MH - 3AcOH]^+$, 567.70 $[MH - 4AcOH]^+$. ^1H NMR (500 MHz, $CDCl_3$), Table 1.

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