Tonkinecin, a Novel Bioactive Annonaceous Acetogenin from *Uvaria tonkinesis*

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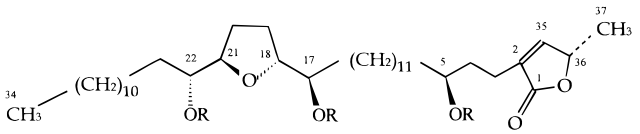
A novel bioactive monotetrahydrofuran acetogenin named tonkinecin (**1**) and two known compounds, uvariamicins I and II, have been isolated from the roots of *Uvaria tonkinesis*. The structure of **1** was elucidated using spectral methods and its absolute stereochemistry established by ^{1}H-NMR experiments utilizing Mosher ester methodology.

Members of the family Annonaceae have recently been investigated as potential sources of biologically active Annonaceous acetogenins; some show a broad spectrum of bioactivity such as antitumor, pesticidal, and other biological activity.[1,2] In the present investigation on *Uvaria tonkinesis* Finet et Gagnep. (Annonaceae), a novel monotetrahydrofuran acetogenin, tonkinecin (**1**), and a mixture of two known compounds, uvariamicins I and II,[3,4] have been isolated from an ethanolic extract of the roots, and the absolute configuration of the new compound **1** has assigned as 5*S*,-17*R*,18*R*,21*R*,22*R*,36*S*. Compound **1** exhibited potent cytotoxicity against the Bel 7402 (hepatoma), BGC (gastrocarcinoma), HCT-8 (colon adenocarcinoma), and HL-60 (leukemia) human tumor cell lines.

Tonkinecin (**1**, Figure 1) was isolated as white crystals (mp 70−72 °C, $[\alpha]^{17}_D$ +26.54°). Its CIMS revealed a $[MH - H_2O]^+$ peak at *m*/*z* 591 indicating a molecular weight of 608 Da. The elemental analysis gave C, 72.87; H, 11.13 (calcd C, 73.02; H, 11.18), corresponding to the molecular formula, $C_{37}H_{68}O_6$. The IR spectrum showed OH group (3441 cm^{-1}) absorption. The presence of three OH moieties was suggested by successive losses of three H_2O molecules (*m*/*z* 18) in the CIMS as well as by the formation of a tri-TMSi derivative **1a**. A prominent IR carbonyl absorption at 1743 cm^{-1} suggested the presence of an α,*β*-unsaturated *γ*-lactone. The NMR spectra of **1** showed ^{1}H-NMR resonances at *δ* 7.02 (q, H-35), 4.99 (dq, H-36), 3.58 (m, H-5), 2.40 (m, H-3), and 1.40 (d, H-37) (Table 1) and six ^{13}C-NMR resonances at *δ* 173.99 (C-1), 149.38 (C-35), 134.08 (C-2), 77.49 (C-36), 70.87 (C-5), and 19.13 (C-37) (Table 1), which confirmed the presence of an α,*β*-unsaturated *γ*-lactone with a 5-OH moiety identical to those of panalicin[5] and narumicins I and II.[6] The EIMS at *m*/*z* 155 (15%) in **1** and at *m*/*z* 227 (50%) in the tris(trimethylsilyl) (TMSi) derivative **1a** formed by the cleavage of the C-5, C-6 bond also supported the presence of a hydroxyl group at C-5. Even though a peak at *m*/*z* 483 formed by competitive cleavage of the C-4, C-5 bond was not observed in the EIMS of **1**, the peaks at *m*/*z* 465 (2%) [*m*/*z* 483 − H_2O] and 447 (2%) [*m*/*z* 483 − 2H_2O] supported the C-4, C-5 cleavage, followed by losses of one and two molecules of H_2O (Figure 2).

The presence of a mono-THF ring in **1**, with two OH groups at the adjacent carbons of the ring, was deduced by the ^{1}H-NMR resonances at *δ* 3.79 (H-18 and H-21) and 3.39 (H-17 and H-22) (Table 1) and ^{13}C-NMR peaks



1 R = H
1a R = Me_3Si
1b R = *S*-MTPA
1c R = *R*-MTPA

Figure 1. Structures of tonkinecin (**1**) and three derivatives. The absolute stereochemistry of the carbinol centers was determined by Mosher ester methodology.

Table 1. ^{1}H- and ^{13}C-NMR Chemical Shifts (ppm) for **1**[a]

proton(s)	δ_H	δ_C
1		173.99
2		134.08
3	2.40 m	21.49
4	1.65 m	35.34
5	3.58 m	70.87
6	1.35 m	37.51
7−15	1.25 br	22.65−31.89
16	1.35 m	33.51
17, 22	3.39 m	74.13
18, 21	3.79 m	82.66
19, 20	1.96, 1.66 m	28.73
23	1.35 m	33.51
24−33	1.25 br	22.65−31.89
34	0.87 t (6.8)	14.05
35	7.02 d (1.4)	149.38
36	4.99 dq (6.8, 1.4)	77.49
37	1.40 d (6.8)	19.13

[a] In $CDCl_3$ at 500 MHz; *J* values (Hz) in parentheses.

at *δ* 82.66 (C-18), 82.66 (C-21), 74.13 (C-17), and 74.13 (C-22) (Table 1), which are characteristic for mono-THF acetogenins having two OH groups adjacent to the ring.[1,2] The number of carbons between the unsaturated lactone and the THF ring was established by EIMS analysis of the fragmentation of the TMSi derivative **1a** (Figure 2). From the abundant ion signals at *m*/*z* 483 and 553, both of which contain the unsaturated lactone ring, it was obvious that the THF ring is located between C-17 and C-22 in the molecule of **1**.

On the basis of the spectral data described above, the three OH groups in **1** were assigned at the C-5, C-17, and C-22 positions. The relative stereochemistry C-17, C-18 and C-21, C-22 was defined by comparing the ^{13}C-NMR signals of the hydroxylated carbons at C-17 (*δ* 74.13) and C-22 (*δ* 74.13) and the ^{1}H-NMR signals of H-17, H-22 (*δ* 3.39) and H-18, H-21 (*δ* 3.79) in **1**, with those of model compounds of known relative stereochemistry.[7] These data suggested that the relative configurations between C-17, C-18 and C-21, C-22 were

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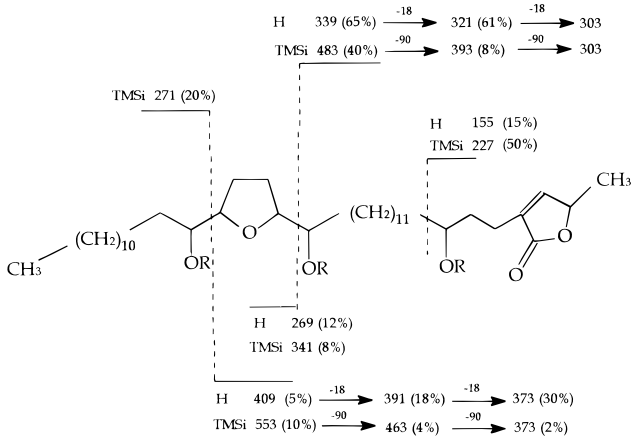

Figure 2. Diagnostic EIMS fragments ions (m/z) of tonkinecin (**1**) and the tonkinecin−TMS derivative **1a**. The m/z 18, m/z 90 ions are indicative of the losses of H_2O and TMSiOH, respectively.

Table 2. ^{1}H-NMR Data of the (*S*)- (**1b**) and (*R*)-MTPA (**1c**) Esters of **1**[a]

proton(s)	(*S*)-MTPA of **1** (**1b**) δ_H	(*R*)-MTPA of **1** (**1c**) δ_H	$\Delta(\delta_S - \delta_R)$
3	2.32 (m)	2.20 (m)	positive
4	1.95−1.20	1.95−1.17	
5	5.11 (m)	5.11 (m)	*S*[b]
6−16	1.95−1.20	1.95−1.17	
17	4.98 (m)	5.05 (m)	*R*[b]
18	3.94 (m)	4.02 (m)	negative
19, 20	1.95−1.20	1.95−1.17	
21	3.94 (m)	4.02 (m)	negitive
22	4.98 (m)	5.05 (m)	*R*[b]
23−33	1.95−1.20	1.95−1.17	
34	0.90 (t, 6.8)	0.90 (t, 6.8)	
35	7.05 (d, 1.4)	6.98 (d, 1.4)	positive
36	5.02 (dq, 1.5, 6.8)	5.00 (dq, 1.5, 6.8)	positive
37	1.41 (d, 6.8)	1.40 (d, 6.8)	positive
MeO	3.55 (s)	3.56 (s)	
MeO	3.55 (s)	3.56 (s)	
MeO	3.55 (s)	3.56 (s)	
Ar	7.61−7.38	7.61−7.37	

[a] In $CDCl_3$ at 500 MHz; multiplicities and *J* vlaues (Hz) in parentheses. [b] Absolute configuration of chiral center.

both threo. The ^{1}H-NMR signals at δ 1.96 and 1.65 corresponding to H-19 and H-20 are typical methylene proton signals of a *trans*-THF ring, since the methylene proton signals for *cis*-THF rings are δ 1.94 and 1.82.[8] Thus, the relative configuration for these four chiral centers in **1** was assigned as threo,trans,threo.

The absolute stereochemistry of the carbinol stereocenters in tonkinecin (**1**) has been determined using Mosher ester methodology[9] based on the differences between the ^{1}H-NMR chemical shifts of (*S*)- and (*R*)-MTPA methoxytrifluoromethylphenylacetate ester derivatives.[9−11] The ^{1}H-NMR data for tonkinecin−(*S*)-MTPA (**1b**) and tonkinecin−(*R*)-MTPA (**1c**) derivatives are summarized in Table 2. According to the Mosher arguments, C-17 and C-22 were assigned with the *R* absolute configuration, since the signs of $\Delta\delta_H(\delta_S - \delta_R)$ were negative for H-18 and H-21 showing relatively less shielding for this side in the (*S*)-MTPA ester. As the relative stereochemistry from C-17 to C-22 of compound **1** is threo,trans,threo, the absolute configurations of C-17(*R*), C-18(*R*), C-21(*R*), and C-22(*R*) were readily determined. With the C-5 carbinol configuration assigned as *S*, the MTPA ester derivative of tonkinecin (**1**) clearly showed the expected sign of the $\Delta\delta_H$ values for H-3 (positive) and H-35−H-37 (positive) (Table 2).[10] The large positive magnitude of the $\Delta\delta_H$ value for H-35 is particularly diagnostic. The configuration at C-36 was assumed as *S* based on the fact that the configuration of this chiral center has been determined to be *S* in most of the acetogenins whose absolute stereochemistry has been solved. Thus, the absolute configuration of tonkinecin (**1**) is proposed as illustrated in Figure 1.

Table 3. Cytotoxicity of Tonkinecin (**1**) for Cancer Cell Lines[a]

compd	HL-60[b]	BGC[c]	HCT-8[d]	Bel 7402[e]
1	0.52	5.1	0.38	1.5
etoposide[f]	0.16	6.7	6.4	1.9

[a] Data are expressed as IC_{50} values (μM). [b] Human leukemia. [c] Human gastrocarcinoma. [d] Human colon adenocarcinoma. [e] Human hepatoma. [f] Positive control.

Two known acetogenin compounds, uvariamicins I and II, were obtained as a mixture. The IR and ^{1}H- and ^{13}C-NMR spectra showed the characteristics of an α,β-unsaturated γ-lactone, one THF ring, two hydroxyl groups, and a long aliphatic chain. NMR studies could not distinguish between the two positional isomers present in the mixture because of their identical chemical shifts. Mass spectral studies established that it was a mixture containing two positional isomers, with the spectral data (IR, MS, ^{1}H- and ^{13}C-NMR) identical to those reported in the literature for uvariamicins I and II.[3,4]

Tonkinecin (**1**) was significantly cytotoxic for human tumor cells in culture (Table 3); it exhibited potent cytotoxicity especially against the HCT-8 (human colon adenocarcinoma) and HL-60 (human leukemia) cell lines.

Experimental Section

General Experimental Procedures. Melting points were determined on a micromelting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter, and IR spectra were run on a Perkin-Elmer 683 infrared spectrometer. The ^{1}H- and ^{13}C-NMR spectra were taken on a Bruker AM 500 spectrometer with TMS as internal standard, and chemical shifts were recorded in δ units. EIMS and CIMS were obtained on a ZAB-2F mass spectrometer. Elemental analyses were determined on a MOD.1106 elemental analyzer.

Plant Material. Roots of *U. tonkinesis* were collected from Longjin County, Guangxi Province, People's Republic of China, in July 1994. The identification was confirmed by Professor Sho-Xiang Liu, Department of Medicinal Plants, Guangxi College of Traditional Chinese Medicine, where a voucher specimen has been deposited.

Extraction and Isolation. The dried and pulverized roots (9.2 kg) were extracted exhaustively with 95% EtOH and the solvent removed to yield extract F001 (950 g) which was partitioned between H_2O and $CHCl_3$ (1:1), giving a water-soluble extract F002 (140 g), a $CHCl_3$-soluble extract F003 (220 g), and insoluble interfacial material F004 (590 g). F003 was partitioned further between petroleum ether and 90% MeOH and yielded the MeOH extract F005 (90 g) and the petroleum ether-soluble extract F006 (115 g). Tonkinecin (**1**) and a mixture of uvariamicins I and uvariamicins II were isolated and purified by chromatography of F005 over Si gel (gradients of petroleum ether/Me_2CO).

Tonkinecin (1): white crystals (200 mg); mp 70−72 °C; $[\alpha]_D$ +26.54° (*c* 0.09, $CHCl_3$); IR *ν* max (KBr) 3441, 2920, 2851, 1743, 1709, 1469, 1074; EIMS (70 eV), see Figure 2; CIMS (isobutane) *m/z* $[MH - H_2O]^+$ (8), $[MH - 2H_2O]^+$ 573 (30), $[MH - 3H_2O]^+$ 555 (36), 537 (6); ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$), see Table 1. Anal. Calcd for $C_{37}H_{68}O_6$: C, 73.02; H, 11.18. Found: C, 72.87; H, 11.13.

TMS Derivative of 1. A small amount (<1 mg) of **1** was treated with *N*,*O*-bis(trimethylsilyl)acetamide/pyridine (10:1) and heated at 70 °C for 30 min to yield the tris-TMSi derivative [**1a**]: EIMS, see Figure 2.

(*R*)- and (*S*)-Mosher Esters of 1. To acetogenin **1** (10 mg, in 1 mL of CH_2Cl_2) were added 4-(dimethylamino)pyridine (DMAP, 5 mg), 80 mg of (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (*R*-MTPA), or (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (*S*-MTPA) and 1,3-dicyclohexylcarbodiimide (DCC, 60 mg); the resulting mixture was stirred at room temperature for 6 h (a white precipitate was formed after several minutes). The reaction mixture was filtered, and the filtrate was concentrated and chromatographed over a Si gel microcolumn (eluted with 0 → 50% Me_2CO in petroleum ether) to give the purified Mosher esters **1b** and **1c**, respectively: ^{1}H-NMR (500 MHz, $CDCl_3$) data of **1b** and **1c**, see Table 2.

Uvariamicins I and II: white amorphous powder (50 mg); mp 59−60 °C; characterized by spectral (IR, EIMS, ^{1}H- and ^{13}C-NMR) analysis and comparison with literature data.[3,4]

Bioassays. Cytotoxicity against human solid tumor cells was measured in 5-day MTT tests at the Department of Pharmacology, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China, using HL-60 leukemia, BGC gastrocarcinoma, HCT-8 colon adenocarcinoma, and Bel 7402 hepatoma cell lines, with etoposide (VP-16) as a positive control (Table 3).

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