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SIX ACETOGENINS FROM *UVARIA TONKINESIS*

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Key Word Index—*Uvaria tonkinesis*; Annonaceae; acetogenin; tonkinins A, B and C; tonkinesins A, B and C.

Abstract—From the root of *Uvaria tonkinesis*, six novel monotetrahydrofuran (THF) annonaceous acetogenins, tonkinins A, B and C and tonkinesins A, B and C, have been isolated and purified. Their structures, characterized by the presence of either a ketone or a hydroxyl group at C-5, were established on the basis of spectral evidence. The occurrence of two isomeric pairs, tonkinins A/B and tonikinesins A/B, is noteworthy. Tonkinin C and tonkinesin C are mono-THF type acetogenins with an unusual flanking acetoxy group adjacent on one side of the THF ring. The absolute stereochemistry of tonkinin C was revealed by the use of Mosher's methodology.

INTRODUCTION

In the last 13 years, bioactive acetogenins characterized by the presence of an α,β-unsaturated γ-lactone and tetrahydrofuran (THF) ring have been isolated from various Annonaceae [1–3]. The compounds act by inhibiting complex I (NADH-Q reductase) of mitochondrial electron transport systems [2], and this action probably accounts for their potent antitumour, pesticidal and other biological activity. From extracts of the root of *Uvaria tonkinesis*, we have isolated six new acetogenins, tonkinins A (**1**), B (**2**) and C (**3**), and tonkinesins A (**4**), B (**5**) and C (**6**). Their structures were determined by NMR, mass spectrometry and analysis of their chemical derivatives. They all have a substituted group at C-5. A thorough comparison of the diagnostic NMR chemical shifts with those of similar known compounds led to the assignments of their relative stereochemistry in the mono-THF part. They all showed selective and potent cytotoxicities to human tumour cell lines.

RESULTS AND DISCUSSION

Compound **1** on CI mass spectrometry gave a $[MH - H_2O]^+$ at m/z 605, indicating a M_r of 622. The molecular formula was established to be $C_{37}H_{66}O_7$ on the basis of elemental analysis and the mass and NMR spectral data. The presence of hydroxyl moieties was obvious from the loss of H_2O (m/z 18) from the molecular ion in the CI mass spectrum and a broad absorption in the IR spectrum at 3357 cm^{-1}. The existence of three hydroxyls was further confirmed by three successive losses of H_2O from the molecular ion in the CI mass spectrum and also by the formation of triacetyl and tri-TMSI (trimethylsilyl) derivatives. A prominent IR carbonyl absorption at 1751 cm^{-1} suggested the presence of an α,β-unsaturated γ-lactone group. Typical resonances in the ^1H NMR at δ 7.05 (*d*, $J = 1.5$ Hz), 4.98 (*dq*, $J = 7.0$ and 1.5 Hz) and 1.39 (*d*, $J = 7.0$ Hz) and the ^{13}C NMR at δ 173.46, 150.45, 132.59, 77.47 and 19.02 supported this conclusion [1, 2]. In addition to the resonances due to the oxygenated carbons of the lactone, the ^{13}C NMR spectrum showed five resonances at δ 83.12, 82.28, 71.83, 71.53 and 68.62, also due to oxygen-bearing carbons. By comparison of these ^{13}C NMR data and their corresponding ^1H NMR resonances at δ 3.89 (3H), 3.80 (1H) and 3.73 (1H) with the NMR signals of known acetogenins, we deduced that **1** had two flanking hydroxyl groups adjacent to a THF ring and one isolated hydroxyl group on the hydrocarbon chain. The IR spectrum of **1** showed an additional strong carbonyl absorption at 1714 cm^{-1}, suggesting the presence of ketone group. This was confirmed by the presence of a carbonyl resonances at δ 209.45.

Compound **2**, in keeping with it having the same carbon skeleton as **1**, gave the same EI mass fragmentation pattern as **1**. The diagnostic fragment ions observed in the EI mass spectrum of **1** and **2** are summarized in Figs 1 and 2, respectively. The NMR spectral data showed that these two isomers differed only in the relative stereochemistry of the THF portion. The remaining parts of the molecules were found to have identical chemical shifts.

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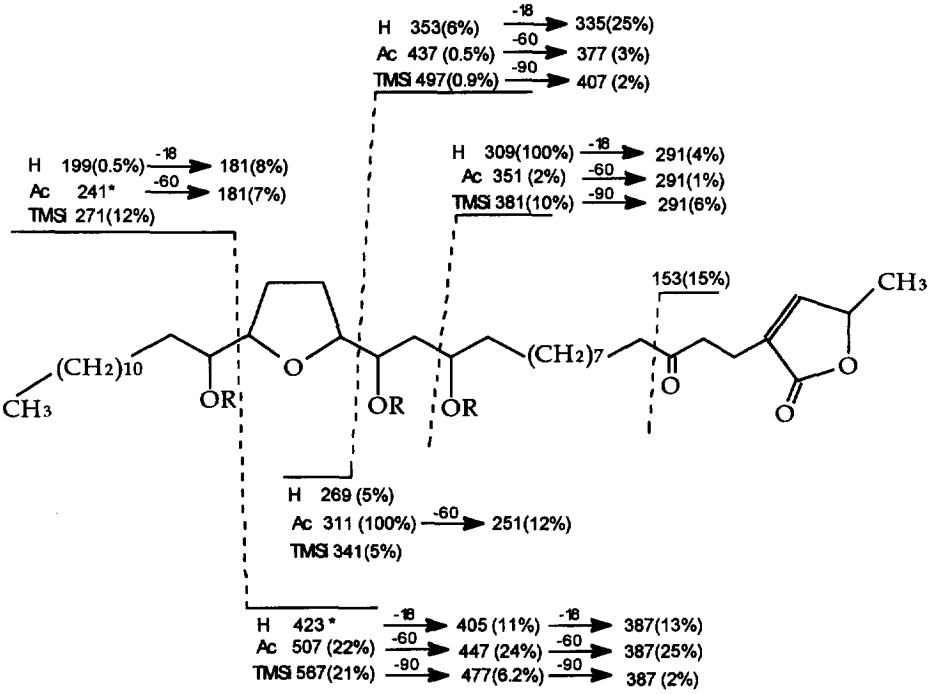


Fig. 1. Diagnostic ions in the EI mass spectrum of tonkinin A (**1**) and tonkinin B(**2**). R = H in the underivatized material, Ac is the acetyl derivative and TMSI is the trimethylsilyl derivative. Ions at *m/z* 18, 60 and 90 are indicative of the loss of H_2O, AcOH and TMSiOH, respectively. *Peak not observed.

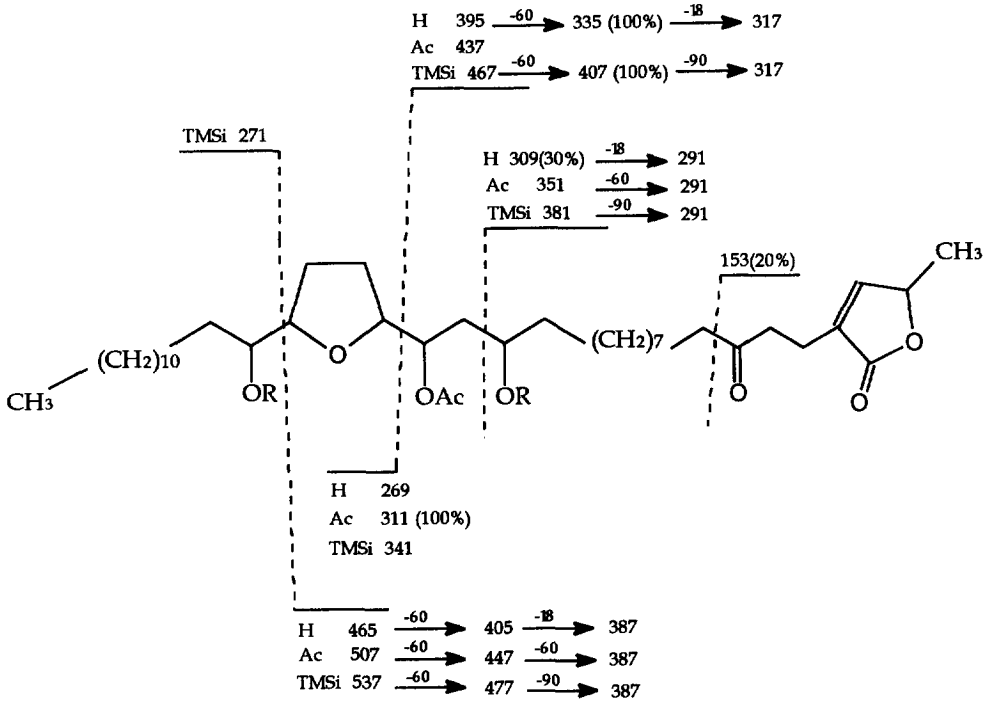


Fig. 2. Diagnostic ions in the EI mass spectrum of tonkinin C (**3**). R = H in the underivatized material, Ac is the acetyl derivative and TMSI is the trimethylsilyl derivative. Ions at *m/z* 18, 60 and 90 are indicative of the loss of H_2O, AcOH and TMSiOH, respectively.

The EI mass spectral ions at *m/z* 153 in the underivatized product and in the tri-TMSi derivative and triacetylated derivative formed by the cleavage of the C-5/C-6 bond supported the presence of a ketone group at the C-5 position. In addition, the EI mass spectrum of both **1** and **2** showed a peak at *m/z* 168 which could easily be explained by the formation of the ion $[CH_2{=}C(OH){-}C_7H_9O_2]^+$ by a McLafferty rearrangement from the $[M]^+$ ion. The formation of such an ion is conclusive proof for the position of the keto group at C-5 [4]. The ^1H NMR data also supported the presence of the ketone at C-5. Thus, two additional two-proton triplets ($J = 7.0$ Hz) were seen in the spectrum of both **1** and **2** at δ 2.71 and 2.38, respectively, consistent with two methylene groups flanking the keto group at C-4 and C-6. Furthermore, in the HMBC spectrum of **2**, the hetero correlations via long-range coupling observed for the atoms in this region [(e.g. δ 209.44 → 2.70 (*m*) (C-5 → H-4), 209.44 → 2.38 (*t*) (C-5 → H-6), 209.44 → 2.56 (*m*) (C-5 → H-3), 173.46 → 2.56 (*m*) (C-1 → H-3), 150.44 → 2.56 (*m*) (C-35 → H-3), 19.46 → 7.04 (*d*) (C-3 → H-35), 132.59 → 2.56 (*m*) (C-2 → H-3), 132.59 → 2.7 (*m*) (C-2 → H-4), 39.75 → 2.56 (*m*) (C-4 → H-3), 19.46 → 2.70 (*m*) (C-3 → H-4), 42.75 → 1.60 (*m*) (C-6 → H-7) and 209.44 → 1.60 (*m*) (C-5 → H-7)] were also in agreement with the position of a keto group at C-5. Moreover, the ^1H and ^{13}C NMR chemical shifts for the atoms at C-3, C-4, C-5 and C-6 in both **1** and **2** were found to be different from the NMR chemical shifts reported for these atoms in other common compounds which possess either a hydroxyl group at C-4 or C-5 or lack any hydroxyl substitution in this region.

The placements of the THF ring and hydroxyl groups were determined by careful analysis of the EI mass fragments of **1** and its acetyl and TMSi derivatives (Fig. 1). The EI mass fragments of **2** are similar to those of **1** (Fig. 1). The EI mass spectrum of the TMSi derivative of **1** produced intense ions at *m/z* 271, 341, 497 and 567 and corresponding signals in the EI mass spectrum of **1**, which clearly placed the THF ring at C-18 along the hydrocarbon chain and allowed the assignment of the hydroxyl groups at C-17 and C-22 relative to the THF ring. The position of the remaining isolated hydroxyl group at C-15 was illustrated by a fragment in the EI mass spectrum of **1** and its acetyl and TMSi derivatives at *m/z* 309, 351 and 381, respectively. These fragments showed losses of H_2O, acetic acid and TMSi hydroxide, respectively, to give *m/z* 291. In the HMBC spectrum of **2**, the hetero correlations observed [δ 71.49 → 1.60 (*m*) (C-17 → H-16), 68.46 → 1.60 (*m*) (C-15 → H-16), 37.56 → 3.81 (*m*) (C-16 → H-15) and 37.56 → 3.76 (*m*) (C-16 → H-17)] confirmed the placement of the free hydroxyl at C-15.

As mentioned above, the spectral data for **1** and **2** suggested that the two acetogenins were isomers with an identical carbon skeleton which differed in the relative configuration in the THF part. The ^1H and ^{13}C NMR chemical shifts of the remaining parts of the molecules, i.e. carbons 1–16, 23–34 and 35–37, were found to be similar for both isomers. A thorough comparison with the diagnostic NMR chemical shifts of a pair of model mono-THF compounds with adjacent hydroxyl groups in the *threo* and *erythro* configuration [1, 2] enabled us to interpret the ^1H and ^{13}C NMR spectra of the two compounds and led to the assignment of the relative stereochemistry in the mono-THF part in both isomers. The relative stereochemistry between C-21 and C-22 of **1** was determined as *erythro* by comparing the ^{13}C NMR signal of **1** for C-22 (δ 71.83 or 71.53) and the ^1H NMR resonances of **1** for H-21 (δ 3.89) and H-22 (δ 3.89) with those of model compounds of known relative stereochemistry [5]. By the technique of Born *et al.* [5], the ^{13}C NMR chemical shift of **1** for C-17 at δ 71.53 or 71.83 suggested the *erythro* relationship between C-17 and C-18. However, considering the *γ-gauche* effect due to the presence of a hydroxyl group substituted at C-15, the ^{13}C NMR chemical shift of C-17 should be shifted upfield. Therefore, the *threo*-relationship between C-17/C-18 was assigned. This is supported by agreement of the assignment determined with that of X-ray crystallographic data for uvarigrin [6]. In addition, the ^{13}C NMR shift of **1** for C-15 upfield shifted to δ 68.62 also supported the presence of a *γ-gauche* effect due to a hydroxyl group at the *β*-position; as the carbons having a single isolated hydroxyl group are typically displayed at δ 70–72 in other acetogenins [1, 2]. According to the method of Jossang *et al.* [7], the small difference value of carbon resonances between C-18/C-21 ($\Delta\delta <$ 1.5 ppm) indicated the *trans* relationship (C-18 → H-17) and 82.18/82.63 → 3.41 (*m*) (C-21 → H-22) and placed the C-17 carbon chemical shift at δ 71.49 and the corresponding carbinol methine proton at δ 3.76, while the C-22 carbon chemical shift was at δ 74.07 and the corresponding carbinol methine proton at δ 3.41. The ^{13}C NMR chemical shift of C-18 and C-21 at δ 82.63 indicated the *trans* relationship between C-18 and C-21 [7]. As in **1**, the relative stereochemistry between C-17 and C-18 of **2** was deduced as *threo*, but the relative configuration between C-21 and C-22 of **2** was also determined as *threo* by comparing the ^1H and ^{13}C NMR signals of **2** for H-22 and C-22 with those of model compounds of known relative stereochemistry. Thus, the isomer named tonkinin A (**1**) was ascribed the relative stereochemistry *threo*, *trans*, *erythro* and the second isomer named tonkinin B (**2**) was ascribed *threo*, *trans*, *threo* going from C-17 to C-22.

Compound **3** was assigned the molecular formula $C_{39}H_{68}O_8$ by elemental analysis (calc. C, 70.48; H, 10.24; found: C, 70.62; H, 10.20]. The ^1H and ^{13}C NMR spectra (Tables 1 and 2) exhibited signals characteristic of mono-THF acetogenins, and, as with **1** and **2**, the existence of an *α,β*-unsaturated *γ*-lactone fragment with a ketone at C-5 was indicated. The presence of an acetoxy group was suggested by the IR carbonyl absorption band at 1723 cm^{-1}, the proton signals at δ 2.05 (3H, *s*, $OCOCH_3$) and the carbon signals at δ 171.60 and 21.16. The existence of two

Table 1. ^{1}H NMR data for compounds **1–6** (500 MHz, $CDCl_3$) [*J*(Hz) in parentheses]

H	1	2	3*	4*	5	6
3	2.55 *m*	2.56 *m*	2.53 *m*	2.40 *m*	2.42 *m*	2.40 *m*
4	2.71 *m*	2.70 *m*	2.68 *m*	1.62 *m*	1.64 *m*	1.60 *m*
5	—	—	—	3.60 *m*	3.59 *m*	3.55 *m*
6	2.38 *t* (7.0)	2.38 *t* (7.0)	2.37 *t* (7.0)	1.45 *m*	1.45 *m*	1.45 *m*
7	1.54 *m*	1.60 *m*	1.53 *m*	1.25 *br*	1.25 *br*	1.21 *br*
8–13	1.25–1.60 *m*	1.25–1.61 *m*	1.23–1.60 *m*	1.25 *br*	1.25 *br*	1.21 *br*
14	1.25–1.60 *m*	1.25–1.61 *m*	1.23–1.60 *m*	1.50 *m*	1.50 *m*	1.48 *m*
15	3.80 *m*	3.81 *m*	3.36 *m*	3.80 *m*	3.81 *m*	3.36 *m*
16	1.54 *m*	1.60 *m*	1.53 *m*	1.58 *m*	1.61 *m*	1.58 *m*
17	3.73 *m*	3.76 *m*	5.08 *m*	3.73 *m*	3.75 *m*	5.06 *m*
18, 21	3.89, 3.89 *m*	3.88, 3.88 *m*	3.84, 3.84 *m*	3.90, 3.90 *m*	3.88, 3.88 *m*	3.84, 3.84 *m*
19, 20	1.60–2.00 *m*	1.67, 1.98 *m*	1.61–1.96 *m*	1.70–2.00 *m*	1.71–2.00 *m*	1.67–1.94 *m*
22	3.89 *m*	3.41 *m*	3.84 *m*	3.90 *m*	3.41 *m*	3.84 *m*
23	1.25–1.60 *m*	1.25–1.61 *m*	1.23–1.60 *m*	1.35 *m*	1.35 *m*	1.32 *m*
24–33	1.25–1.60 *m*	1.25–1.61 *m*	1.23–1.60 *m*	1.25 *br*	1.25 *br*	1.22 *br*
34	0.88 *t* (7.0)	0.87 *t* (7.0)	0.85 *t* (6.8)	0.88 *t* (7.0)	0.87 *t* (7.0)	0.85 *t* (6.8)
35	7.05 *d* (1.5)	7.04 *d* (1.5)	7.03 *d* (1.4)	7.03 *d* (1.5)	7.04 *d* (1.5)	7.03 *d* (1.4)
36	4.98 *dq* (7.0, 1.5)	4.98 *dq* (7.0, 1.5)	4.96 *dq* (6.8, 1.5)	5.00 *dq* (7.0, 1.5)	4.98 *dq* (7.0, 1.5)	4.96 *dq* (6.8, 1.5)
37	1.39 *d* (7.0)	1.37 *d* (7.0)	1.37 *d* (6.8)	1.40 *d* (7.0)	1.37 *d* (7.0)	1.37 *d* (6.8)
AcO	—	—	2.05 *s*	—	—	2.05 *s*

*The assignments were made on the basis of ^{1}H–^{1}H COSY.

hydroxyl moieties was determined by two successive losses of H_2O from $[M - \text{acetic acid}]^+$ in the EIMS. This was confirmed by the preparation of a diacetate derivative (**3a**) and a di-TMSi derivative (**3b**), and by IR and NMR data. The ^{1}H (Table 1) and ^{13}C NMR (Table 2) data for **3** showed the existence of a mono-THF ring with two adjacent substituent groups. The positions of the THF ring and hydroxyl acetoxy and moieties along the aliphatic chain were determined by the EI mass spectral analysis of **3** and its TMSi derivative **3b** (Fig. 2). The EI mass spectrum of **3b** produced intense ions at *m/z* 271, 341, 381, 467 and 537, which clearly indicated the position of the THF ring at C-18 and the assignment of the two hydroxyl groups at C-15 and C-22, and the acetoxy group at C-17. This was in agreement with the corresponding signals in the EI mass spectra of **3** and **3a**. The ^{1}H–^{1}H COSY spectrum of **3** showed that both H-15 (3.36, *m*)

Table 2. ^{13}C NMR spectral data for compounds **1–6** (125 MHz, $CDCl_3$)

C	1	2	3*	4*	5	6
1	173.46	173.46	173.45	173.78	173.62	174.07
2	132.59	132.59	132.58	134.07	134.03	133.92
3	19.47	19.46	19.46	21.50	21.48	21.09
4	39.75	39.75	39.75	35.38	35.32	35.22
5	209.45	209.44	209.40	70.83	70.86	70.73
6	42.75	42.75	42.74	37.50	37.46	37.46
7	39.06	39.33	34.93	22.67–31.92	22.64–31.89	22.57–31.81
8–13	22.64–32.55	22.64–33.38	22.64–32.49	22.67–31.92	22.64–31.89	22.57–31.81
14	22.64–32.55	22.64–33.38	22.64–32.49	39.14	39.38	35.18
15	68.62	68.46	70.37	68.78	68.61	70.39
16	37.58	37.56	38.58	37.61	37.56	38.48
17	71.53†	71.49	71.54	71.89	71.56	71.35†
18, 21	83.12, 82.28	82.18, 82.63	82.80, 82.47	83.07–82.28	82.75–82.61	82.79–82.66
19, 20	22.64–32.55	22.64–33.38	28.50, 25.00	22.67–31.92	22.645–31.89	22.57–31.81
22	71.83†	74.07	71.44	71.67	74.10	71.54†
23	22.64–32.55	22.64–33.38	22.64–32.49	32.64	33.47	33.44
22–33	22.64–32.55	22.64–33.38	22.64–32.49	22.67–31.92	22.64–31.89	22.57–31.81
34	14.07	14.06	14.07	14.08	14.06	13.98
35	150.45	150.44	150.43	149.44	149.47	149.48
36	77.47	77.46	77.46	77.55	77.56	77.53
37	19.02	19.02	19.02	19.16	19.12	19.02
AcO	—	—	21.16, 171.60	—	—	21.39, 171.57

*The assignments were made on the basis of ^{13}C–^{1}H COSY.
†May be interchanged.

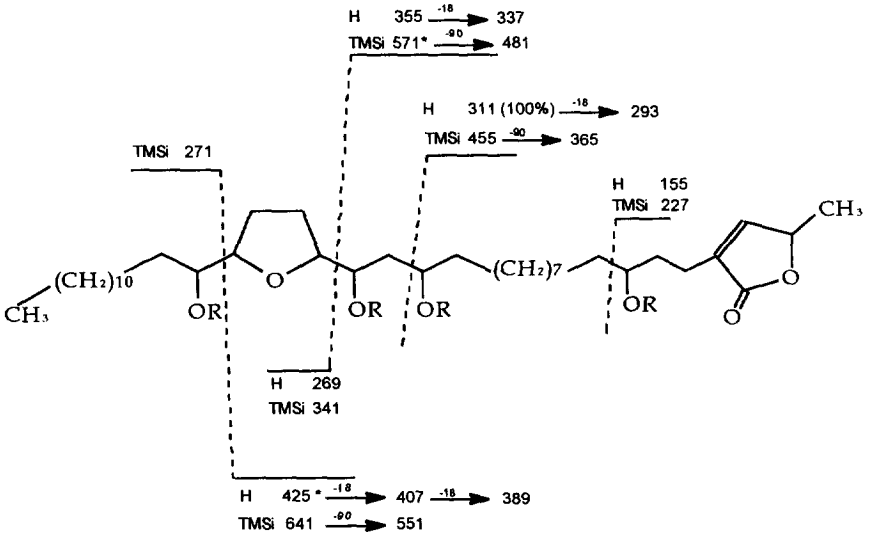


Fig. 3. Diagnostic ions in the EI mass spectrum of tonkinesin A (**4**) and tonkinesin B (**5**). R = H in the underivatized material and TMSi is the trimethylsilyl derivative. Ions at *m/z* 18 and 90 are indicative of the loss of H_2O and TMSiOH, respectively. *Peak not observed.

and H-17 (5.08, *m*) correlated with H-16 (1.53, *m*). This observation confirmed the position of the free hydroxyl at C-15.

A thorough comparison with the diagnostic HMR chemical shifts of the similar compound uvarigrin [6] and **1** and **2** led us to the assignment of the relative stereochemistry in the THF part of **3**. According to Jossang's method, the small difference value of carbon resonances between C-18/C-21 ($\Delta\delta < 1.5$ ppm) indicated the *trans* relationship between C-18 and C-21; the ^{13}C NMR chemical shift of C-22 at δ 71.44 clearly showed the relative configuration between C-21 and C-22 was *erythro* [3, 5]; as in 1, the relative stereochemistry between C-17 and C-18 of **3** was determined as *threo*. The ^1H NMR data for **3** also supported the determination of a *threo*, *trans*, *erythro* relationship from C-17 to C-22, according to Hoye and co-workers' method [8, 9].

Mosher's methodology was recently demonstrated by Rieser and co-workers [10, 11] to be a valuable method for the determination of the absolute stereochemistry of the carbinol chiral centres in acetogenins. The (*S*)- and (*R*)-methoxylfluoromethylphenylacetic acid (MTPA) esters (Mosher esters) of **3** (**3c** and **3d**) were prepared and their proton chemical shifts were carefully assigned according to the ^1H–^1H COSY data (Table 3). The analyses of the $\Delta\delta_H(\delta_S-\delta_R)$ of H-23 gave a negative result on the side chain. According to Mosher's assumptions [12–14], only the *S* configuration of C-22 could have the protons on the side chain showing relatively more high shielding in the (*S*)-MTPA derivative, and vice versa in the (*R*)-MTPA ester. Thus, the *S* configuration was assigned for the carbinol centre at C-22. As the relative stereochemistry from C-17 to C-22 of **3** was *threo*, *trans*, *erythro*, the absolute configuration of C-17 (*R*), C-18 (*R*), C-21 (*R*) and C-22 (*S*) was thus readily concluded. The $\Delta\delta_H$ showing positive data for H-16, H-17 and H-18 and negative data for H-14, suggested that the configuration at C-15 was *S*, based on the same arguments as used above. The configuration at C-36 was assumed to be *S* based on the fact that the configuration of this chiral centre has been determined to be *S* in most of acetogenins whose absolute stereochemistries have been solved [5, 9, 15–17]. The absolute configuration of **3** was, therefore, concluded to be 15*S*, 17*R*, 18*R*, 21*R*, 22*S* and 36*S*.

Compound **4** was isolated as a white powder. Its CI mass spectrum showed $[M+H]^+$ at *m/z* 625. A molecular formula of $C_{37}H_{68}O_7$ was established on the basis of elemental analysis and mass and NMR spectral data. Diagnostic mass spectrum fragmentation, as well as comparison of its ^1H and ^{13}C NMR spectral data with known compounds, suggested that **4** belongs to the class of bioactive mono-THF acetogenins. The existence of four hydroxyl groups in the structure was deduced from the fragment ions of *m/z* 607, 589, 571, and 553 in the CI mass spectrum, which arise by

Table 3. ^1H NMR spectral data for **3**-*S* and -*R*-MTPA ester [$\delta(J$ = Hz)]*

H	**3**-*S*-MTPA	**3**-*R*-MTPA	$\Delta(\delta S - \delta R)$
14	1.54	1.56	Negative
15	4.84	4.62	*S*
16	1.75	1.62	Positive
17	5.15	5.07	Positive
18	3.90	3.76	Positive
19	1.87, 1.58	1.75, 1.52	Positive
20	1.78, 1.72	1.72, 1.66	Positive
21	3.97	3.98	*ca* Zero
22	5.24	5.27	*S*
23	1.52	1.58	Negative

*The assignments were made on the basis of COSY ^1H–^1H NMR.

consecutive losses of four H_2O molecules and from the formation of a tetra-TMSi derivative. The locations of the THF ring and other hydroxyl groups were determined by careful analysis of the EI mass spectrum of the TMSi derivative of **4** as depicted in Fig. 3. Based on the above data, **4** was found to be a 5-hydroxyl analogue of **1**. The CI mass spectral ions at *m/z* 155 (22%) in **1** and at *m/z* 227 (30%) in the tetra-TMSi detivative formed by a cleavage of the C-5/C-6 bond supported the location of a hydroxyl group at C-5, as in the case of the reported compounds panalicin [4] and narumicins I and II [18]. A comparison of the NMR spectral data for **4** with that for **1** also supported the fact that it is a hydroxyl derivative of **1**. In the ^1H NMR spectrum, the signals at δ 2.71 and 2.38 consistent with two methylene groups flanking the ketone group at C-4 and C-6 in **1** were missing in **4** and an additional one proton multiplet of H-5 at δ 3.60 was seen in the spectrum of **4**. Moreover, the ^{13}C chemical shifts of C-3 to C-7 are also in agreement with the position of the hydroxyl group at C-5. The relative stereochemistry of the mono-THF portion was, therefore, suggested to be *threo*, *trans*, *erythro* from C-17 to C-22 on the basis of the close similarity of the ^{13}C NMR data for **4** and **1**.

Compound **5** was obtained as white powder. The EI mass fragmentation pattern of **4** and **5** established the in identical carbon skeletons. The diagnostic fragment ions observed in the EI mass spectrum of **4** and **5** are summarized in Fig. 3. The NMR spectral data showed that these two acetogenins were isomers with an identical carbon skeleton which differed only in the relative configuration of the THF portion. Comparison of diagnostic carbon chemical shifts of **5** with **2** showed that they have identical relative stereochemistry in the mono-THF part, thus confirming compound **5** as a 5-hydroxy analogue of **2**.

Compound **6**, was obtained as an oil. Its CI mass spectrum displayed $[MH]^+$ at *m/z* 667. The elemental analysis gave C, 70.45; H, 10.42 (calc. C, 70.27; H, 10.51). The ^1H and ^{13}C NMR spectra (Table 1 and 2) exhibited signals characteristic of mono-THF acetogenins and an α,β-unsaturated γ-lactone moiety with a hydroxyl group at C-5. The presence of an acetoxy group was suggested by the proton signals at δ 2.03 (3H, *s*, $OCOCH_3$) and the carbon signals at δ 171.57 and 21.39. The existence of three hydroxyl groups was established by three successive losses of H_2O from $[M - \text{acetic acid}]^+$ in the EI mass spectrum. This was confirmed by preparation of a tri-TMSi derivative **6a**, and by the IR and NMR data. The ^1H (Table 1) and ^{13}C NMR (Table 2) data for **6** showed the existence of a mono-THF ring with two adjacent substituent groups. The position of the THF ring, and hydroxyl and acetoxy moieties along the aliphatic chain were determined based on the EI mass spectral analyses of **6** and its TMSi derivative **6a** (Fig. 4). The fragment ions at *m/z* 227, 271, 341, 455 and 481 (541 − acetic acid), and 551 (611 − acetic acid) in the EI mass spectrum of **6a** clearly indicated the position of the THF ring at C-18 and the assignment of an acetoxy at C-17, and three hydroxyl groups at C-5, C-15 and C-22. This was in agreement with the corresponding signals in the EI mass spectrum of **6**. The *threo*, *trans*, *erythro* structure at the mono-THF portion of **6** was deduced from a comparison of the ^{13}C NMR data for **6** with those for **1** and **3**.

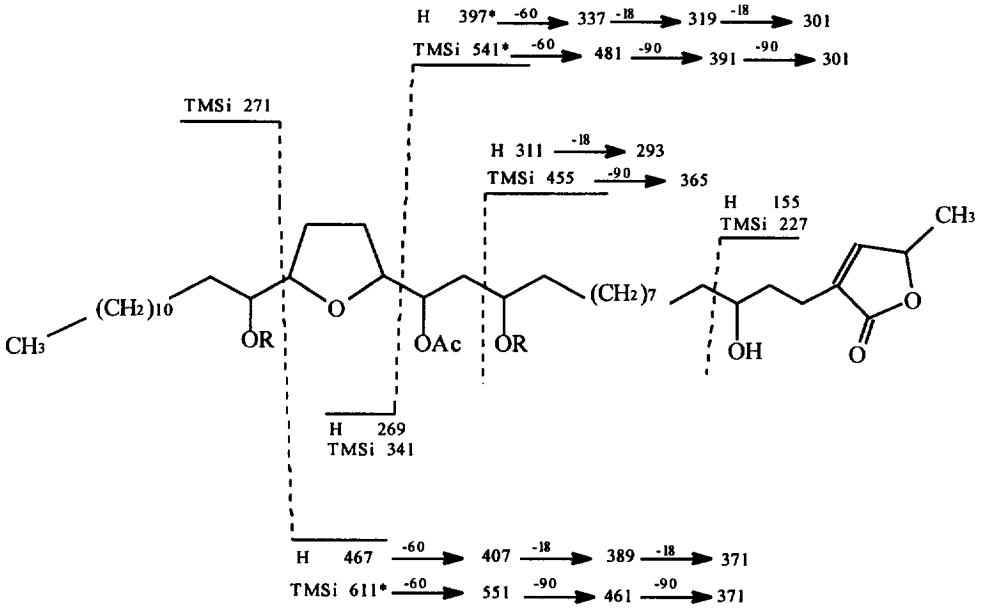
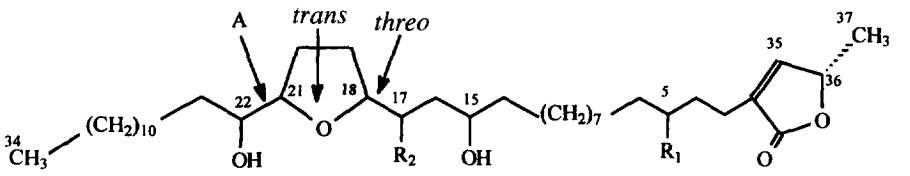
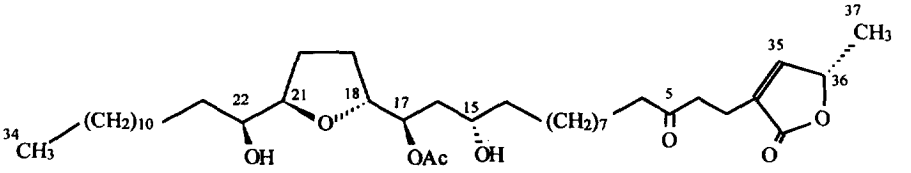


Fig. 4. Diagnostic ions in the EI mass spectrum of tonkinesin C (**6**). R = H in the underivatized material and TMSi is the trimethylsilyl derivative. Ions at *m/z* 18 and 90 are indicative of the loss of H_2O and TMSiOH, respectively. *Peaks not observed.



	R_1	R_2	A
1	=O	OH	*erythro*
2	=O	OH	*threo*
4	OH	OH	*erythro*
5	OH	OH	*threo*
6	OH	OAc	*erythro*



3

The biological activities of **4**, **5** and **6** are summarized in Table 4. Compounds **4**–**6** were potently and selectively cytotoxic to human tumour cells. Compound **6** showed selectivity towards human leukaemia (HL-60) and human colon adenocarcinoma (HCT-8) versus human nasopharyngeal carcinoma (KB) and human breast carcinoma (A 2780) and was especially potent to HL-60. Compounds **5** and **6** also exhibited selective cytotoxicity in HL-60. Compound **6** is a 17-acetyltonkinesin A; its activity towards human leukaemia (HL-60) was stronger than that of **4**. These observations are of interest for the structure–activity relationship of annonaceous acetogenins.

EXPERIMENTAL

Mps: uncorr.; IR: KBr; ^{1}H NMR, ^{13}C NMR and HMBC: Bruker AM500 spectrometer in $CDCl_3$; CIMS and EIMS: ZAB-2F spectrometer by desorption chemical ionization using isobutane.

Plant material. Root of *U. tonkinesis* was collected from Guang Xi Province, China, in July 1994. Identifi-

Table 4. Bioactivities of compounds **4**, **5** and **6**

Compound	HCT-8 IC_{50} (μM)	HL-60 IC_{50} (μM)	KB IC_{50} (μM)	A 2780 IC_{50} (μM)
1	5.9×10^{-1}	1.6×10^{-2}	>10	>10
2	2.44	6.0×10^{-2}	>10	>10
3	8.6×10^{-1}	4.15×10^{-4}	>10	>10
VP-16	6.7	0.13	1.9	6.4

HCT-8 = Human colon adenocarcinoma.
HL-60 = Human leukaemia.
KB = Human nasopharyngeal carcinoma.
A 2780 = Human breast carcinoma.
VP-16 = Etoposide, positive control.

cation was confirmed by Prof. Shou Xiang Liu, Department of Medicinal Plants, Guang Xi College of Traditional Chinese Medicine, where a voucher specimen has been deposited.

Extraction and purification. Air-dried pulverized root (9.2 kg) was extracted exhaustively with 95% EtOH and evapd *in vacuo* to yield extract F001 (950 g), which was partition between H_2O and $CHCl_3$ (1:1), giving a water-soluble fr, F002 (140 g), a $CHCl_3$-soluble fr, F003 (220 g) and an insoluble interface, F004 (590). F003 was further partitioned between petrol and 90% MeOH and yielded the MeOH fr. F005 (90 g) and the petrol fr., F006 (115 g). Further purification of F005 by repeated chromatography over silica gel (gradients of $CHCl_3$–MeOH and petrol–Me_2CO) gave **1**, **2**, **3**, **4**, **5** and **6**.

Tonkinin A (**1**). Crystals (280 mg), mp 98–100°. $[\alpha]_D^{16}$ +20.09° ($CHCl_3$, *c* 0.112). IR ν_{max}^{KBr} cm^{-1}: 3357 (OH), 2919, 2849, 1751 (C=O), 1714 (C=O), 1070; CI-MS (isobutane), *m/z*: 605 $[MH-H_2O]^+$, 587 $[MH-2H_2O]^+$, 569 $[MH-3H_2O]^+$, 309; EI-MS *m/z*: Fig. 1; EI-MS (triacetates and tri-TMSi derivative) *m/z*: Fig. 1; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$): Tables 1 and 2. Anal. calc. for $C_{37}H_{66}O_7$: C, 71.38; H, 10.61 (found: C, 71.29; H, 10.68). *Triacetyltonkinin A*: Treatment of **1** (micro-amount) with Ac_2O–pyridine (1:1) at room temp. for 48 hr. EI-MS: Fig. 1. *Tri(trimethylsilyl)tonkinin A*: Dry micro-amount samples of **1** were treated with *N,O*-bis(trimethylsilyl)acetamide (BSA) and pyridine (10:1) and heated at 70° for 30 min, EI-MS: Fig. 1.

Tonkinin B (**2**). Waxy solid (110 mg), mp 95–96°. $[\alpha]_D^{16}$ +28.20° ($CHCl_3$, *c* 0.098). IR ν_{max}^{KBr} cm^{-1}: 3410 (OH), 2920, 2849, 1738 (C=O), 1711 (C=O), 1068, 1029; EI-MS *m/z* (rel. int.): 153 (12), 168 (13), 181 (8), 269 (8), 291 (6), 309 (100), 335 (30), 353 (6), 387 (20), 405 (12%), 423 (1.6) similar to **1**: Fig. 1. Anal. calc. for $C_{37}H_{66}O_7$: C, 71.38; H, 10.61 (found: C, 71.54; H, 10.70).

Tonkinin C (**3**). Amorphous powder (1250 mg), mp 40–42°. $[\alpha]_D^{16}$ +10.80° ($CHCL_3$, *c* 0.119). IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 2919, 2850, 1759, 1723, 1702, 1468, 1377, 1255, 1077, 1030; EI-MS *m/z* (rel. int.): 153 (20), 168 (15), 181 (10), 269 (9), 335 (100), 351 (2), 387 (20), 395 (4), 405 (18), 447 (3), 461 (2), 465 (8), 479 (3), 568 (3) $[M-HOAc-2H_2O]^+$, 586 (4) $[M-HOAc-H_2O]^+$: Fig. 2; ^1H NMR 500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$): Tables 1 and 2. Anal. calc. for $C_{39}H_{68}O_8$: C, 70.48; H, 10.24 (found: C, 70.62; H, 10.20). *Diacetyltonkinin C*: Treatment of **3** (micro-amount) with Ac_2O–pyridine (1:1), room temp. for 48 hr. EI-MS: Fig. 2. *Di(trimethylsilyl)tonkinin C*. Dry micro-amount samples of **3** were treated with BSA and pyridine (10:1) and heated at 70° for 30 min. EI-MS: Fig. 2.

R- *and* S-*Mosher esters*. To **3** (10 mg in 1 ml CH_2Cl_2) was added 4-(dimethylamino)pyridine (5 mg), 50 mg (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (*R*-MTPA) or (*S*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (*R*-MTPA) or (*S*)-(−)-α-methoxy-α-(trifluoromethyl) phenylacetic acid (*S*-MTPA) and 1,3-dicyclohexylcarbodiimide (50 mg). The resulting mixt. was stirred at room temp. for 6 hr (a ppt. was formed after several min). The reaction mixt. was filtered, and the filtrate was concd and subjected to CC on a silica gel microcolumn (eluted with 0→50% Me_2CO in petrol) to give purified Mosher esters **3c** and **3d**, respectively. ^1H NMR (500 MHz, $CDCl_3$): Table 3.

Tonkinesin A (**4**). Powder (16 mg), mp 97–99°. $[\alpha]_D^{20}$ +26.92° ($CHCl_3$, *c* 0.026). IR ν_{max}^{KBr} cm^{-1}: 3379.1 (OH), 2918.1, 2850.6, 1741.6 (C=O); CI-MS (isobutane) *m/z*: 625 $[MH]^+$, 607 $[MH-H_2O]^+$, 589 $[MH-2H_2O]^+$, 553 $[MH-4H_2O]^+$, 463, 407, 389, 371, 355, 337, 319, 311, 293, 281, 269, 199, 181, 155; EI-MS *m/z*: Fig. 3. ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$): Tables 1 and 2. Anal. calc. for $C_{37}H_{68}O_7$: C, 71.15; H. 10.90 (found: C, 71.68; H, 10.52). *Tetra(trimethylsilyl)tonkinesin A*: Compound **4** was treated as described for the prepn of tri-TMSi tonkinin A. EI-MS: Fig. 3.

Tonkinesin B (**5**). Powder (10 mg), mp 80–81°. $[\alpha]_D^{20}$ +24.49° ($CHCl_3$, *c* 0.049). IR ν_{max}^{KBr} cm^{-1}: 3433.1 (OH), 2920.1, 2850.6, 1747.4 (C=O), 1082.0; EI-MS *m/z* (rel. int.): 155 (8), 269 (18), 293 (60), 311 (70), 319 (30), 337 (35), 355 (10), 371 (15), 289 (20), 407 (10), 425 (1), 553 $[MH-4H_2O]^+$, 571 $[MH-3H_2O]^+$, 589 $[MH-2H_2O]^+$, $[MH-2H_2O]^+$, 607 $[MH-H_2O]^+$, 625 $[MH]^+$; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$): Tables 1 and 2.

Tonkinesin C (**6**). Oil (20 mg), $[\alpha]_D^{20}$ +25.19° ($CHCl_3$, *c* 0.651). IR ν_{max}^{KBn} cm^{-1}: 3465.9 (OH), 2923.9, 2852.6, 1737.8, 1245.9; CI-MS *m/z* (rel. int.): 153 (10), 269 (10), 293 (20), 301 (10), 319 (18), 11 (35), 337 (50), 371 (21), 389 (21), 397 (4), 407 (15), 449 (5), 467 (10), 553 (23) $[M-HOAc-3H_2O]^+$, 571 (52) $[M-HOAc-2H_2O]^+$, 589 (100) $[M-HOAc-H_2O]^+$, 607 (82) $[MH-HOAc]^+$, 631 (8) $[MH-2H_2O]^+$, 649 (30) $[MH-H_2O]^+$, 667 (60) $[MH]^+$; EIMS *m/z*: Fig. 4. ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$): Tables 1 and 2; Anal. calc. for $C_{39}H_{70}O_8$: C, 70.27; H, 10.51 (found: C, 70.40; H, 10.42). *Tri(trimethylsilyl)tonkinin C*. Compound **6** was treated as described for the prepn of tri-TMSi tonkinin A.

Bioassay. Cytotoxicity against human solid tumour cells was measured in 5-day MTT tests at the Department of Pharmacology, Institute of Materia Medica, for the HL-60 leukaemia, HCT-8 colon adenocarcinoma, KB nasopharyngeal carcinoma, A 2780 breast carcinoma cell lines, with Etoposide (VP-16) as a positive control.

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