Pergamon

PII: S0031-9422(97)00153-2

Phytochemistry, Vol. 45, No. 7, pp. 1423–1426, 1997

0031-9422/97 $17.00+0.00

ANNONISIN, A BIS-TETRAHYDROFURAN ACETOGENIN FROM *ANNONA ATEMOYA* SEEDS*

PHILIPPE DURET,† REYNALD HOCQUEMILLER and ANDRÉ CAVÉ

Laboratoire de Pharmacognosie, URA 1843 CNRS (BIOCIS), Faculté de Pharmacie, Université Paris-Sud, 92296 Châtenay-Malabry Cedex, France

(*Received in revised form* 20 *January* 1997)

Key Word Index—*Annona atemoya*; Annonaceae; seeds; acetogenins; annonisin.

Abstract—A new C_{35} tetrahydroxy adjacent bis-tetrahydrofuran acetogenin, annonisin, has been isolated from the methanol extract of *Annona atemoya* seeds and characterized by spectroscopic techniques, in addition to the known compounds, molvizarin, parviflorin, rolliniastatin-1, asimicin, motrilin, cherimolin-1, cherimolin-2, almunequin and annonacin. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Acetogenins from Annonaceae are known to exhibit a variety of pharmacological activities, i.e. parasiticide, insecticide, cytotoxic antitumoural, and immunosuppressive [2]. In a continuation of our studies on this family, we have investigated the acetogenins from the seeds of *Annona atemoya* (cv African Pride), a hybrid between *Annona squamosa* L. and *Annona cherimolia* Mill., and cultivated for its edible fruits in Australia. We have recently described two new monotetrahydrofuran acetogenins from the seeds which we named annotemoyins -1 and -2 [3]. In the present paper, we report on the isolation and structure elucidation of annonisin (**1**), a new adjacent bis-tetrahydrofuran acetogenin with 35 carbons from the methanol extract of *A. atemoya* seeds. Nine additional acetogenins [2]: molvizarin (**2**) [4], parviflorin (**3**) [5], rolliniastatin-1 (**4**) [6], asimicin (**5**) [7], motrilin (**6**) [4], cherimolin-1 (**7**) [8], cherimolin-2 (**8**) [8], almunequin (**9**) [8] and annonacin (**10**) [9], have also been obtained. Whereas **2**, **3**, **5**, **6**, **7** and **9** have been previously reported [10], **4**, **8** and **10** were isolated for the first time in this plant.

RESULTS AND DISCUSSION

Annonisin (**1**) gave an $[M+H]^+$ peak at *m/z* 611 on CI mass spectroscopy (methane). The molecular formula was deduced as $C_{35}H_{62}O_8$ by HRCI mass spectroscopy which gave the $[M+H]^+$ ion at *m/z* 611.4513 (calcd 611.4523). The presence of four hydroxyl groups was indicated by the sequential loss of four molecules of H_2O from the $[M+H]^+$ ion. This was further confirmed by a broad absorption in the IR spectrum at 3432 cm^{-1} and by preparation of the tetraacetyl derivative **1a** the CI-mass spectrum of which showed the successive loss of four AcOH units from the molecular peak (*m/z* 779). The presence in annonisin of an α,β-unsaturated γ-lactone with an hydroxyl group at C-4 was suggested by a positive response to Kedde's reagent, a weak UV absorption maximum at 212 nm, a strong IR absorption at 1748 cm^{-1}, and the ^1H NMR resonances at δ 7.19 (H-33), 5.05 (H-34), 3.84 (H-4), 2.50 (H-3a), 2.38 (H-3b) and 1.42 (H-35) corresponding in the ^{13}C NMR to resonances at δ 151.8 (C-33), 77.9 (C-34), 69.6 (C-4), 33.3 (C-3) and 19.1 (C-35) (Table 1) [2]. Peaks at *m/z* 141 and 213 in the EI-mass spectra of **1** and its tetra-TMSi-derivative (**1b**), respectively (cleavage between C-4 and C-5), supported this assignment.

The presence of an adjacent bis-THF part was verified by the ^1H NMR signal at δ 3.83 assigned to four protons attached to methine carbons bearing oxygen (H-14, H-17, H-18, H-21) in agreement with their ^{13}C NMR signals at δ 83.2 (C-14, C-21) and 81.8 (C-17, C-18). A proton resonance at δ 3.38 (H-13, H-22) was assignable to two protons attached to secondary hydroxylated carbons observed at δ 74.1 (C-13, C-22) in the ^{13}C NMR spectrum of annonisin. 2D experiments (COSY-DQF, HMQC and HMBC) confirmed that these hydroxyl groups were on each side of the bis-THF system (**1**). The signals in the ^1H and ^{13}C NMR spectra of **1** at δ 3.59 and 71.4 were characteristic of an hydroxyl group isolated on the alkyl chain [2].

The locations of the α,α'-bis-THF system as well

* Part 55 in the series 'Acetogenins from Annonaceae'. For part 54 see ref. [1].

† Author to whom correspondence should be addressed.

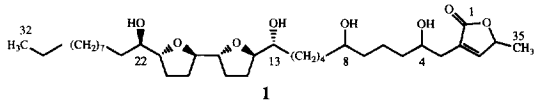
Table 1. ^{1}H NMR and ^{13}C NMR data for annonisin (**1**) and ^{1}H NMR resonances for its tetraacetyl derivative (**1a**) ($CDCl_3$, δ)

Position	^{1}H NMR (**1**)*	^{13}C NMR (**1**)	Annonisin tetraacetate (**1a**)
1	—	174.6	—
2	—	131.2	—
3a	2.50 *dd* (15.2, <1.0)	33.3	2.53 *m*
3b	2.38 *ddd* (15.2, 8.2, 1.4)	33.3	
4	3.84 *m*	69.6	5.09 *m*
5	1.47 *m*	37.2	1.20–1.97
6	1.25–1.60 *m*	21.6	1.20–1.97
7	1.43 *m*	37.2	1.20–1.97
8	3.59 *m*	71.4	4.84 *m*
9	1.43 *m*	37.2	1.20–1.97
10–11	1.25–1.60 *m*	22.5–29.4	1.20–1.97
12	1.43 *m*	33.3	1.20–1.97
13	3.38 *m*	74.1	4.84 *m*
14	3.83 *m*	83.2	3.97 *m*
15–16	1.63 *m*, 1.97 *m*	28.4, 29.6	1.43–1.97
17–18	3.83 *m*	81.8	3.90 *m*
19–20	1.63 *m*, 1.97 *m*	28.4, 29.6	1.43–1.97
21	3.83 *m*	83.2	3.97 *m*
22	3.38 *m*	74.1	4.84 *m*
23	1.43 *m*	33.3	1.20–1.97
24–29	1.25–1.60 *m*	22.5–29.4	1.20–1.97
30	1.25 *m*	31.9	1.43–1.97
31	1.25 *m*	22.6	1.43–1.97
32	0.86 *t* (6.8)	14.0	0.88 *t* (6.3)
33	7.19 *d* (1.3)	151.8	7.08 *d* (1.1)
34	5.05 *dq* (6.8, <1.0)	77.9	5.01 *dq* (7.0, 1.6)
35	1.42 *d* (6.8)	19.1	1.40 *d* (6.9)
4-OAc	—	—	2.02 *s*
8-OAc	—	—	2.03 *s*
13-OAc	—	—	2.07 *s*
22-OAc	—	—	2.07 *s*

* *J* (Hz) in parentheses.

as the fourth hydroxyl group (Fig. 1) were unambiguously established by careful analysis of the mass spectra of annonisin (**1**), its tetraacetate (**1a**) and its tetra-TMSi-derivatives (**1b**). The peaks at *m/z* 281, 369, 439, *m/z* 425, 495, 565 and *m/z* 515, 585, 655 in the EI-mass spectra of **1**, **1a** and **1b**, respectively, allowed placement of the bis-THF part between C-13 and C-22, whereas the fragment ions at *m/z* 195 for **1**, *m/z* 297 for **1a** and *m/z* 357 for **1b** (C-8/C-9 cleavage) in the EI-mass spectra, suggested that the fourth hydroxyl group was at C-8. This was supported by the upfield shift in the C-6 signal (δ 21.6) due to the two β-effects of the hydroxy-methine carbon atoms 4 and 8.

The relative configuration of the carbon centres of the bis-THF moiety was determined as *threo/trans/threo/trans/threo* (Fig. 1) by comparing the ^{1}H NMR signals of **1a** for H-13 (δ 4.84), H-14 (δ 3.97), H-17 (δ 3.90), H-18 (δ 3.90), H-21 (δ 3.97), H-22 (δ 4.84), 13-OAc (δ 2.07) and 22-OAc (δ 2.07) with those of model compounds of known configuration (Table 1) [11–13]. This assignment was supported by careful comparison of the ^{13}C NMR resonances of **1** with those of parviflorin (**3**) [5] and asimicin (**5**) [7]. Thus, the structure of annonisin (**1**) was determined to be as shown in Fig. 1 and appears to be the first C_{35} bis-THF γ-lactone acetogenin with four hydroxyl groups in positions 4, 8, 13 and 22.



1

Molvizarin (**2**), parviflorin (**3**), rolliniastatin-1 (**4**), asimicin (**5**), motrilin (**6**), cherimolin-1 (**7**), cherimolin-2 (**8**), almunequin (**9**) and annonacin (**10**) were also isolated and their identities were established by comparison of their IR, NMR and MS spectra with those of authentic samples obtained in our laboratory and/or previously reported.

EXPERIMENTAL

General. UV: MeOH; 1D and 2D NMR spectra ($CDCl_3$): 200 and 50 MHz or at 400 and 100 MHz,

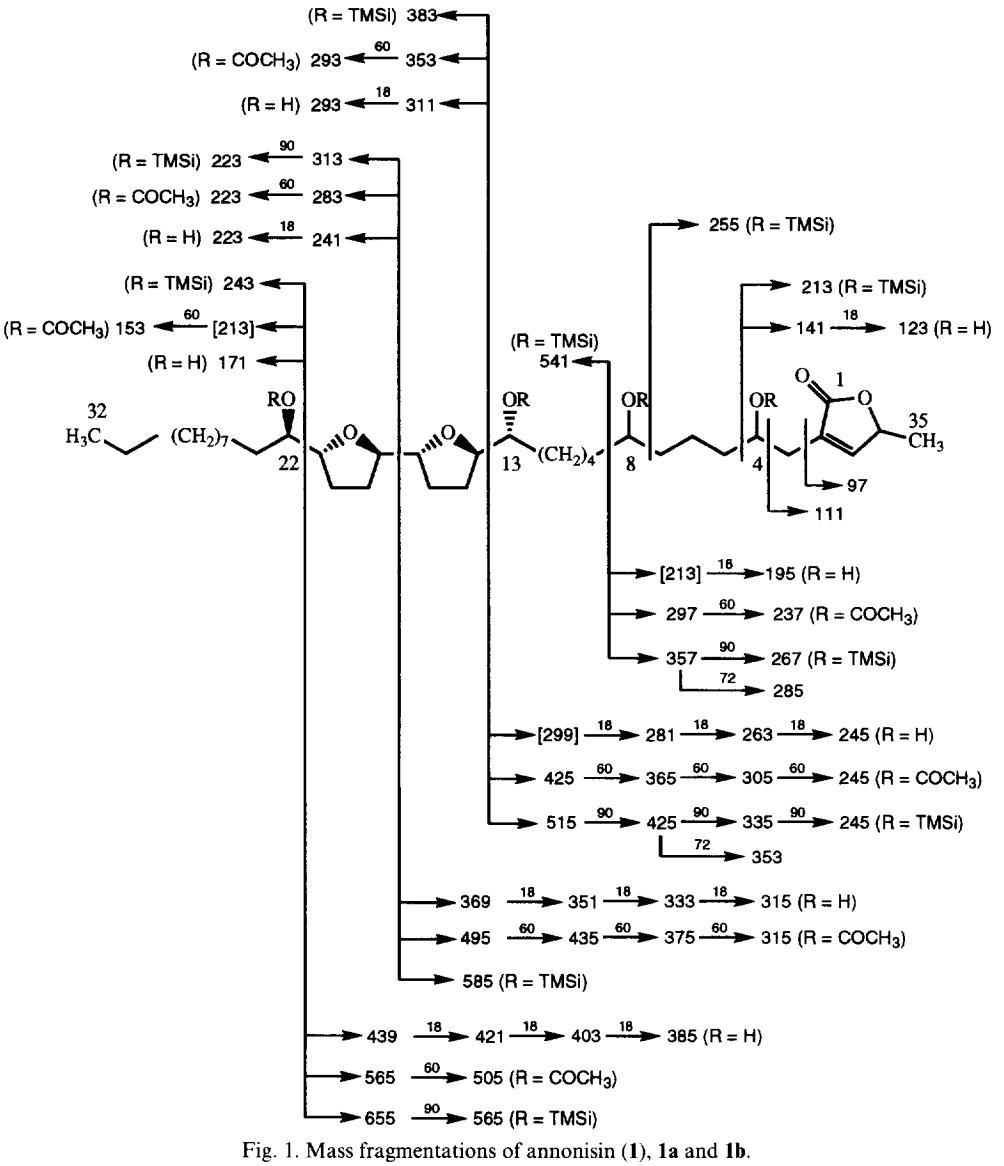


Fig. 1. Mass fragmentations of annonisin (**1**), **1a** and **1b**.

respectively; EIMS and CIMS (methane): Nermag R10-10C spectrometer; HPLC: μBondapak C_{18} prepacked column (10 μm, 8 × 100 mm), elution with 15–20% H_2O–MeOH at various flow rates (0.8–1 ml min^{-1}), with monitoring at 214 nm; Prep. HPLC: μBondapak C_{18} prepacked column (10 μm, 25 × 100 mm), elution with 15–20% H_2O–MeOH at various flow rates (6–10 ml min^{-1}), with monitoring at 214 nm.

Plant material. Seeds of *Annona atemoya* were collected in September 1993 in Australia and identified and authenticated by Dr D. Batten, Tropical Fruit Research Station, Alstonville, New South Wales, Australia.

Extraction and isolation. The dried and pulverized seeds (930 g) were macerated with MeOH. The MeOH extract (79.7 g; LC_{50} < 0.01 μg ml^{-1} in BST [14] was partitioned between H_2O and hexane to yield 6 g of hexane extract (LC_{50} = 0.12 μg ml^{-1} in BST). The aq. alcohol fr. was partially evapd and extracted with CH_2Cl_2. 10.1 g of the CH_2Cl_2-soluble extract (LC_{50} < 0.01 μg ml^{-1} in BST), was fractionated by flash chromatography (elution with toluene–AcOEt–EtOH 6:14:1) giving several frs. Annonisin (**1**) (3.5 mg), molvizarin (**2**) (50 mg), parviflorin (**3**) (18 mg), rolliniastatin-1 (**4**) (6 mg), asimicin (**5**) (18 mg), motrilin (**6**) (23 mg), cherimolin-1 (**7**) (40 mg), cherimolin-2 (**8**) (12 mg), almunequin (**9**) (48 mg) and annonacin (**10**) (7 mg) were obtained as almost pure frs and were further purified by chromatography over silica gel 60 H and/or HPLC.

Annonisin (**1**). White solid (3.5 mg) by prep. HPLC μBondapak C_{18}; MeOH–H_2O (4:1); flow rate 6 ml min^{-1}; R_t 33.4 min. $C_{35}H_{62}O_8$. $[\alpha]_D^{20}$ +30° ($CHCl_3$; *c* 0.20); UV λ_{max}^{MeOH} nm (log ε): 209 (3.9); IR ν_{max}^{NaCl} cm^{-1}: 3432, 2923, 2850, 1748, 1436, 1372, 1068, 1032, 957,

753; CIMS (CH_4) *m/z*: 611 $[MH]^+$ (100%), 593 $[MH-H_2O]^+$, 575 $[MH-2H_2O]^+$, 557 $[MH-3H_2O]^+$, 539 $[MH-4H_2O]^+$, 521, 463, 439, 421, 403, 385, 369, 351, 333, 315, 311, 293, 281, 263, 245, 241, 223, 195, 171, 141, 123, 111; EIMS 40 eV *m/z*: 403, 385, 369, 333, 293, 281, 263, 241, 223, 141, 97 (Fig. 1); ^1H NMR: Table 1; ^{13}C NMR: Table 1. Because of the very limited amount of the sample, no study of the absolute stereochemistry of the chiral centres of **1** was possible.

Annonisin tetraacetate(**1a**). Treatment of **1** (1 mg) with Ac_2O/pyridine (room temp. overnight) and subsequent work-up gave compound **1a** (quantitative yield) as an oil. CIMS (CH_4) *m/z*: 779 $[MH]^+$, 762, 719 $[MH-AcOH]^+$, 659 $[MH-2AcOH]^+$, 599 $[MH-3AcOH]^+$, 539 $[MH-4AcOH]^+$; EIMS 40 eV *m/z*: 719, 659, 609, 565, 547, 505, 495, 435, 425, 375, 365, 353, 315, 305, 297, 293, 283, 245, 237, 223, 171, 153, 123, 111 (Fig. 1); ^1H NMR: Table 1.

Tetra-TMSi-annonisin (**1b**). A small quantity of **1** was treated with 50 μl of BSTFA/1% TMCS and heated at 80° for 20 min to yield its tetra-TMSi-derivative (**1b**). EIMS 37 eV *m/z*: 655, 585, 565, 541, 515, 425, 383, 357, 352, 335, 313, 284, 267, 245, 243, 223, 213 (Fig. 1).

Acknowledgements—This research was sponsored by the 'Direction de la Recherche et des Etudes Doctorales' (DRED), through a biennal contract with the 'Réseau de Recherche Pharmacochimie'. We wish to thank Dr D. Batten (NSW Agriculture, Tropical Fruit Research Station, Alstonville, Australia) for the plant collection, J.-C. Jullian and L. Mascrier for NMR measurements and S. De Barros for the mass spectra.

REFERENCES

1. Raynaud, S., Fourneau, C., Hocquemiller, R., Sévenet, T. and Cavé, A., *Phytochemistry*, (in press).
2. Cavé, A., Figadère, B., Laurens, A. and Cortes, D., *Progress in the Chemistry of Organic Natural Products: Acetogenins from Annonaceae*, Vol. 70, ed. W. Hertz. Springer, Wien, 1997, p. 81.
3. Duret, P., Hocquemiller, R., Cavé, A. and Batten, D., *Natural Product Letters*, 1996, **8**, 89.
4. Cortes, D., Myint, S. H. and Hocquemiller, R., *Tetrahedron*, 1991, **47**, 8195.
5. Ratnayake, S., Gu, Z.-M., Miesbauer, L. R., Smith, D. L., Wood, K. V., Evert, D. R. and McLaughlin, J. L., *Canadian Journal of Chemistry*, 1994, **72**, 287.
6. Pettit, G. R., Cragg, G. M., Polonsky, J., Herald, D. L., Goswami, A., Smith, C. R., Moretti, C., Schmidt, J. M. and Weileder, D., *Canadian Journal of Chemistry*, 1987, **65**, 1433.
7. Rupprecht, J. K., Hui, Y.-H. and McLaughlin, J. L., *Journal of Natural Products*, 1990, **53**, 237.
8. Cortes, D., Figadère, B. and Cavé, A., *Phytochemistry*, 1993, **32**, 1467.
9. McCloud, T. G., Smith, D. L., Chang, C.-J. and Cassady, J. M., *Experimentia*, 1987, **43**, 947.
10. Chen, W.-S., Yao, Z.-J., Xu, Y.-Z., Zhang, Y.-B. and Wu, Y.-L., *Youji Huaxue*, 1996, **16**, 145.
11. Hoye, T. R. and Suhadolnik, J. C., *Journal of the American Chemical Society*, 1987, **109**, 4402.
12. Hoye, T. R. and Zhuang, Z.-P., *Journal of Organic Chemistry*, 1988, **53**, 5578.
13. Ramirez, E. A. and Hoye, T. R., *Studies in Natural Products*, ed. Atta-Ur-Rahman. Elsevier, Amsterdam, 1995, p. 251.
14. Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E. and McLaughlin, J. L., *Planta Medica*, 1982, **45**, 31.