Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 16, pp. 1865-1868, 1995
Elsevier Science Ltd
Printed in Great Britain
0960-894X/95 $9.50+0.00

0960-894X(95)00309-6

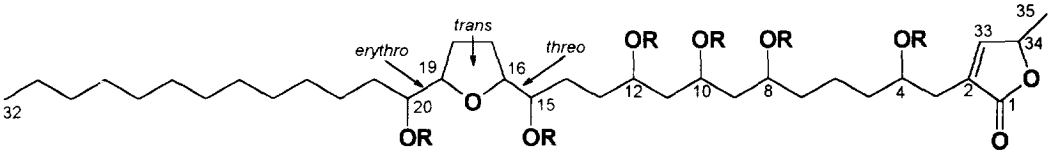
ANNOHEXOCIN, A NOVEL MONO-THF ACETOGENIN WITH SIX HYDROXYLS, FROM *ANNONA MURICATA* (ANNONACEAE)

Lu Zeng, Feng-E Wu, and Jerry L. McLaughlin*

Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907, U. S. A.

Abstract: A novel bioactive acetogenin, annohexocin (**1**), was isolated from the leaves of the sour sop (or guanabana) tree, *Annona muricata* (Annonaceae). Compound **1** is a C-35 mono-tetrahydrofuran (THF) acetogenin having six OH groups, and its structure has been elucidated by spectroscopic analyses. Compound **1** is the first Annonaceous acetogenin to be reported in which six OH groups include a 1,3,5 triol. Compound **1** was isolated by following brine shrimp lethality, and it showed significant inhibitory effects among six human tumor cell lines.

Annona muricata L., known as sour sop (or guanabana), is a popular fruit tree cultivated throughout the tropical regions of the world.[1] Previous reports, studying the seeds, leaves, and twigs, have described several mono-tetrahydrofuran (THF) Annonaceous acetogenins.[2] Further separation of the F005 fraction of the ethanolic extract of the leaves, directed by brine shrimp lethality (BST) for bioactive components,[3] led to a new C-35 mono-THF acetogenin, annohexocin (**1**). Compound **1** has six hydroxyls, three of which are in an unusual 1,3,5-triol arrangement from C-8 to C-12,[4] two are flanking the THF ring from C-15 to C-20, and one is at C-4. The planar structure of **1** was determined by MS, ^{1}H and ^{13}C NMR, HMQC, COSY, and carefully designed single-relayed COSY experiments.



1, R=H **1a**, R=Ac **1b**, R= TMSi

Annohexocin (**1**, white powder, $[\alpha]^{22}_{D}$ +18.5, *c* 0.33 in $CHCl_3$) was separated from the more polar open column chromatographic fractions by repeated reversed-phase and normal-phase HPLC. The molecular weight of **1** was determined by a peak at m/z 629 (MH^+) in the FABMS. The HRFABMS gave m/z 629.6417 (calcd. 629.6428) corresponding to the molecular formula, $C_{35}H_{64}O_9$. The ^{1}H NMR and ^{13}C NMR spectra of **1** showed respective signals at δ 3.45, 74.6, 3.88, 86.2, 1.62, 2.04, 28.5, 1.86, 1.90, 25.2, 3.86, 82.8 and 3.81, 71.5 which suggested that **1** was a typical mono-THF acetogenin bearing two flanking OH groups and having the *threo trans erythro* configuration across these hydroxyls and the THF ring. The presence of an α,β-

unsaturated γ-lactone ring with a 4-OH was suggested by the UV spectrum (λ max 208 nm, log ε 3.24) and the corresponding proton and carbon resonances in the ^{1}H NMR and ^{13}C NMR spectra (Table 1).[4] The formation of a hexaacetate derivative, **1a**, (Table 1) demonstrated the presence of the six OH groups. These groups were also confirmed by inspecting the ^{13}C NMR spectrum, in which six oxygenated carbon signals, at δ 69.7, 71.5, 72.3, 72.3, 73.7, and 74.6, corresponding to the protons at δ 3.89, 3.81, 3.89, 3.95, 4.13, and 3.45, respectively, in an HMQC experiment were observed.

The evidence for the presence of the 1,3,5-triol relationship for three of the OH groups in **1** was provided by COSY and single-relayed COSY experiments. In the COSY spectrum of **1**, cross peaks were found from δ 3.89 to 1.52 then to 4.13, then to 1.54, and then to 3.95. In the single-relayed COSY (Figure 1),[5] cross peaks were observed between δ 3.89 and 4.13, and δ 3.95 and 4.13. The ^{13}C NMR spectrum also showed this relationship, in which two carbon resonances of methylene groups between a 1,3-diol were found at δ 43.4 and 43.5.[4c] The placements of the 1,3,5-triol unit and the THF ring along the hydrocarbon chain were concluded from mass fragmentations of the TMSi derivative, **1b**, the fragment ion at *m/z* 357 showed cleavage between C-8 and C-9, and those at *m/z* 471 and 589 showed cleavage between C-12 and C-13, indicating that the 1,3,5-triol is located at C-8, 10, and 12. These placements were further confirmed by HREIMS, in which the exact masses were determined for the fragments at *m/z* 357.1898 (calc. 357.1917) corresponding to the formula $C_{17}H_{33}O_4Si_2$, the fragment at *m/z* 471.3699 (calc. 471.3690) corresponding to the formula $C_{26}H_{55}O_3Si_2$, and the fragment at *m/z* 499.2746 (calc. 499.2731) corresponding to the formula $C_{24}H_{47}O_5Si_3$ (from the fragment at *m/z* 589, $C_{27}H_{57}O_6Si_4$ with a loss of $C_3H_{10}OSi$). The THF ring was placed at C-16 after observing the fragments at *m/z* 719 and 341, and *m/z* 789 and 271 (Figure 2).

Table 1. ^{13}C NMR (125 MHz) and ^{1}H NMR (500 MHz) Data of **1** and **1a**.

No.	1		1a	No.	1		1a
	δ_C	δ_H (*J* in Hz)	δ_H (*J* in Hz)		δ_C	δ_H (*J* in Hz)	δ_H (*J* in Hz)
1	174.8	-	-	16	86.2	3.88 m	3.96 m
2	130.9	-	-	17	28.5	1.62 m, 2.04 m	1.56 m, 1.94 m
3	33.4	2.42 m, 2.53 m	2.51 m, 2.54 m	18	25.2	1.86 m, 1.90 m	1.74 m, 1.94 m
4	69.7	3.89 m	5.10 m	19	82.8	3.86 m	3.96 m
5	37.1	1.45 m	-*	20	71.5	3.81 m	4.91 m
6	21.3	1.54 m	-*	21	32.5	1.37 m	1.50 m
7	36.9	1.43 m	1.50 m	22	25.9	1.32 m	-*
8	72.3[a]	3.95 m[c]	4.86 m	23-29	29.4-29.7	1.26-1.33 br	1.20-1.35 br
9	43.5[b]	1.54 m[d]	1,75 m, 1.84 m	30	31.9	1.31 br	1.31 br
10	73.7	4.13 dt (2.0, 9.0)	4.80 m	31	22.7	1.26 m	1.26 m
11	43.4[b]	1.52 m[d]	1,75 m, 1.84 m	32	14.1	0.88 t (7.0)	0.88 t (7.0)
12	72.3[a]	3.89 m[c]	4.91 m	35	19.1	1.40 d (6.5)	1.40 d (6.5)
13	34.2	1.60 m	1.52 m	34	78.1	5.06 dq (1.5, 6.5)	5.01 dq (1.5, 6.5)
14	28.6	1.58 m	1.54 m	33	152.3	7.20 q (1.0)	7.08 q (1.0)
15	74.6	3.45 dt (3.0, 7.0)	4.86 m				

[a-d] Assignments may be interchangeable. * Those signals were overlapped in the range of δ1.25-1.70. AcO (**1a**): δ 2.02, 2.04, 2.04, 2.05, 2.05, 2.08.

Relative stereochemistries across the THF ring and flanking hydroxyls were assigned either as *threo/trans/erythro* or *erythro/trans/threo* based on ^{1}H and ^{13}C NMR data which were consistent with model compounds.[6] Whether the *threo* configuration was on the right side or the left side of the THF ring remained in question. Acetogenins with mono-THF rings show the characteristic chemical shift value for the hydroxymethine proton adjacent to the THF ring with the *threo* relationship at *ca.* δ 3.40 (*threo/trans/threo*, δ 3.41, *threo/trans/erythro*, δ 3.40, *threo/trans*, δ 3.37 from model compounds).[4c,6] This is true for the bis-adjacent THF acetogenins in which the corresponding protons appear at δ 3.36-3.41.[4b] When there is another proton nearby, particularly, with the two hydroxylated methines located two carbons apart, the above protons will shift downfield. Examples are found in the structures of goniothalamicin (δ 3.43),[7a] *cis*-goniothalamicin (δ 3.46),[7b] bullatanocin (δ 3.43) and *cis*-bullatanocin (δ 3.44),[7c] 12-hydroxybullatacinones (δ 3.45),[7d] and squamocin F (δ 3.44)[7e]. Compound **1** shows a hydroxymethine proton signal at δ 3.45, indicating that this proton is at C-15, and, thus, the relative stereochemistries across the THF ring and flanking hydroxyls are *threo/trans/erythro*.

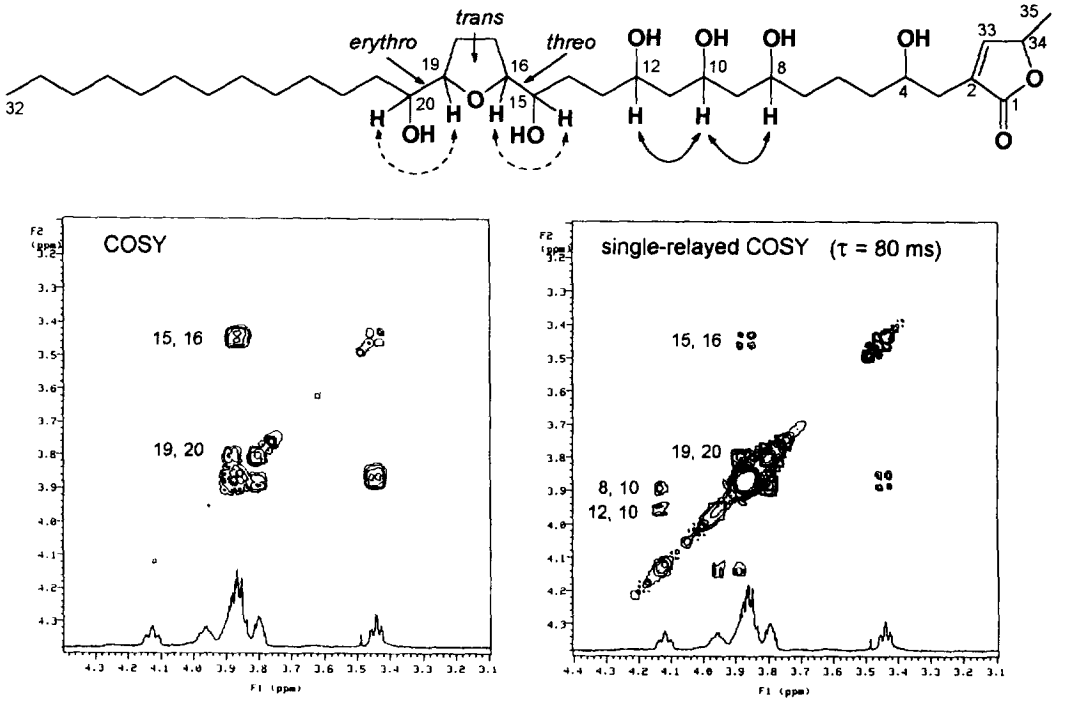


Figure 1. COSY and single-relayed COSY experiments of **1** (the dotted arrows showing the COSY correlations, the solid arrows showing single-relayed COSY correlations).

Compound **1** showed bioactivities in the BST[3] (LC_{50} 34.4 μg/ml) and significant cytotoxicities among six human solid tumor cell lines with selectivity for the prostate cell line [A-549 (lung carcinoma) ED_{50} (μg/ml) 0.34,[8a] MCF-7 (breast carcinoma) 2.26,[8b] HT-29 (colon adenocarcinoma) 0.78,[8c] A-498 (kidney carcinoma) 2.36,[8a] PC-3 (prostate adenocarcinoma) 0.0195,[8d] PACA-2 (pancreatic carcinoma) 0.77].[8c] Adriamycin as a

positive control in the same run gave respective ED_{50} values (μg/ml) of 0.0346, 0.43, 0.0896, 0.0335, 0.33, and 0.0310. The acetogenins act as inhibitors of complex I in mitochondrial electron transport systems and as inhibitors of the plasma membrane NADH oxidase of tumor cells.[9]

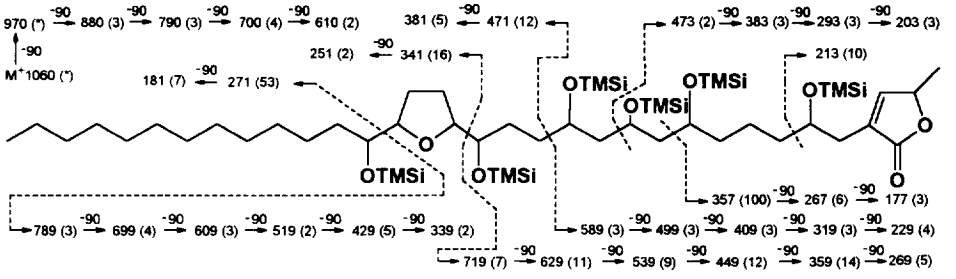


Figure 2. Mass fragmentations of **1b**, the peak intensities are in parentheses (The peaks with asterisks are beyond the limit of observation).

Acknowledgment: This work was supported by R01 grant no. CA30909 from the National Cancer Institute, NIH, and a stipend to FEW from the K. C. Wang Education Foundation, Hong Kong.

REFERENCES AND NOTES

1. Morton, J. *Fruits of Warm Climates*; Media: Florida, 1973; p 65.
2. (a) Rieser, M. J.; Kozlowski, J. F.;Wood, K. V.; McLaughlin J. L. *Tetrahedron Lett.* **1991**, *32*, 1137; (b) Rieser, M. J.; Fang, X.-P.; Rupprecht, J. K.; Hui, Y.-H.; Smith, D. L.; McLaughlin J. L. *Planta Med.* **1993**, *59*, 91; (c) Wu, F.-E; Gu, Z.-M.; Zeng, L.; Zhao, G.-X.; Zhang, Y.; McLaughlin, J. L. *J. Nat. Prod.* **1995**, in press. (d) Wu, F.-E; Zeng, L.; Gu, Z.-M.; Zhao, G.-X.; Zhang, Y.; Schwedler, J. T.; McLaughlin, J. L. *J. Nat. Prod.* **1995**, in press; (e) Wu, F.-E; Zeng, L.; Gu, Z.-M.; Zhao, G.-X.; Zhang, Y.; Schwedler, J. T.; McLaughlin, J. L. *J. Nat. Prod.* **1995**, in press; (f) Wu, F.-E; Zhao, G.-X.; Zeng, L.; Zhang, Y.; Schwedler, J. T.; McLaughlin, J. L. *J. Nat. Prod.* **1995**, in press, (g) Zeng, L.; Wu, F.-E; Gu, Z.-M.; McLaughlin J. L. *Tetrahedron Lett.* **1995**, in press; (h) Myint, S. H.; Laurens, A.; Hocquemiller, R.; Cavé, A. *Heterocycles* **1990**, *31*, 861; (i) Roblot, F.; Laugel, T.; Leboeuf, M.; Cavé, A.; Laprevote, O. *Phytochemistry* **1993**, *34*, 281 and references cited therein.
3. Meyer, B. N.; Ferrigni, N. R.; Putnam, J. E.; Jacobson, L. B.; Nichols, D. E.; McLaughlin, J. L. *Planta Med.* **1982**, *45*, 31; . McLaughlin, J. L. In *Methods in Plant Biochemistry*; Hostettmann, K. Ed.; Academic: London, 1991; Vol 6, p 1.
4. (a) Rupprecht, J. K.; Hui, Y.-H.; McLaughlin, J. L. *J. Nat. Prod.* **1990**, *53*, 237; (b) Fang, X.-P.; Rieser, M. J.; Gu, Z.-M.; Zhao, G.-X.; McLaughlin, J. L. *Phytochem. Anal.* **1993**, *4*, 27; (c) Gu, Z.-M.; Zhao, G.-X.; Oberlies, N. H.; Zeng, L.; McLaughlin, J. L. In *Recent Advances in Phytochemistry*; Romeo, J. T. Ed.; Plenum: New York, 1995; Vol 29, (in press); (d) Cavé, A.; Cortes, D.; Figadere, B.; Hocquemiller, R.; Laprevote, O.; Laurens, A.; Leboeuf, M., In *Recent Advances In Phytochemistry*; Downum, K. R.; Romeo, J.; Stafford, H. A., Eds.; Plenum: NewYork, 1993; Vol 27, p 167.
5. (a) Eich, G.; Bodenhausen, G.; and Ernst, R. R. *J. Am. Chem. Soc.* **1982**, *104*, 3731, (b) Bax, A. and Drobny, A. *J. Magn. Reson.* **1985**, *61*, 306.
6. (a) Fujimoto, Y.; Murasaki, C.; Shimada, H.; Nishioka, S.; Kakinuma, K.; Singh, S.; Singh, M.; Gupta, Y. K.; Sahai, M. *Chem. Pharm. Bull.* **1994**, *42*, 1175; (b) Harmange, J. C.; Figadere, B.; Cavé, A. *Tetrahedron Lett.* **1992**, *33*, 5749.
7. (a) Alkofahi, A.; Rupprecht, J. K.; Smith, D. L.; Chang, C.-J.; McLaughlin, J. L. *Experientia* **1988**, *44*, 83; (b) Rieser, M. J. Ph.D. Thesis. Purdue University, West Lafayette, Indiana, 1993; (c) Gu, Z. M.; Zeng, L.; McLaughlin, J. L. *Heterocycles* **1995**, *41*, 229; (d) Gu, Z.-M.; Fang, X.-P.; Hui, Y.-H.; McLaughlin J. L. *Natural Toxins* **1994**, *2*, 49-55; (e) Sahai, M.; Singh, S.; Singh, M.; Gupta, Y. K.; Akashi, S.; Yuji, R.; Hirayama, K.; Asaki, H.; Araya, H.; Hara, N.; Eguchi, T.; Kakinuma, K.; Fujimoto, Y. *Chem. Pharm. Bull.* **1994**, *42*, 1163.
8. (a) Giard, D. J.; Aaronson, S. A.; Todaro, G. J.; Arnstein, P.; Kersey, J. H.; Dosik, H.; Parks, W. P. *J. Natl. Cancer Inst.* **1973** *51*, 1417; (b) Soule, H. D.; Vazquez, J.; Long, A.; Albert, S.; Brennan, M. *J. Natl. Cancer Inst.* **1973**, *51*, 1409; (c) Fogh, J.; Trempe, G. In *Human Tumor Cell*; Fogh, J. Ed., Plenum: New York, 1975; p 115; (d) Kaighn, M. E.; Narayan, K. S.; Ohnuki, Y.; Lechner, J. F.; Jones, L. W. *Invest. Urol.* **1979**, *17*, 16; (e) Yunis, A. A.; Arimura, G. K.; Russin, D. *Int. J. Cancer* **1977**, *19*, 128
9. (a) Ahammadsahib, K. I.; Hollingworth, R. M.; McGovren, J. P.; Hui, Y.-H.; McLaughlin, J. L. *Life Sci.* **1993**, *53*, 1113; (b) Morre, D. J.; de Cabo, R.; Farley, C.; Oberlies, N. H.; McLaughlin, J. L. *Life Sci.* **1995**, *56*, 343.

(*Received in USA* 1 *June* 1995; *accepted* 14 *July* 1995)