Chlorinated Annonaceous Acetogenins and Their Bioactivities

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Received June 11, 1996[⊗]

4(*S*)-Chloro-4-deoxygigantetrocin A and 4(*S*),18-dichloro-4,18-dideoxyasimilobin were obtained by treating gigantetrocin A with triphenylphosphine and CCl_4. The structures were determined by spectroscopic methods. The chlorinated compounds show decreased bioactivities in the brine shrimp lethality test and against human tumor cell lines.

The annonaceous acetogenins are a class of promising anticancer, antiinfective, and pesticidal natural products.[1−5] These compounds typically have zero to three tetrahydrofuran (THF) rings, a number of oxygenated moieties in the middle of the long hydrocarbon chains, and terminal lactones. So far, more than 220 annonaceous acetogenins have been reported;[4] most of the oxygenated moieties are found to be free hydroxyls. Until now, no chlorinated acetogenins have been prepared. To test how chlorination might affect the bioactivities of the acetogenins, two chlorinated products, 4(*S*)-chloro-4-deoxygigantetrocin A (**1**) and 4(*S*),18-dichloro-4,18-dideoxyasimilobin (**2**), were prepared and tested in the brine shrimp lethality test (BST)[6,7] and against six human solid tumor cell lines. The results showed that chlorination decreased the bioactivities but indicated some cytotoxic selectivities.

Triphenylphosphine (TPP) and CCl_4 are useful for the chlorination and dehydration of primary and secondary alcohols.[8] Gigantetrocin A[9,10] was refluxed with TPP and CCl_4 for 16 h. After separation by HPLC, compound **1** was obtained as a colorless wax. The CIMS gave two molecular ion peaks at *m*/*z* 614 and 616, with the peak ratio of 3:1, suggesting the presence of one chloro atom in the molecule. Comparing the spectral data of **1** with that of gigantetrocin A, **1** showed the characteristic features for the α,*β*-unsaturated *γ*-lactone moiety, a mono-THF ring with one flanking hydroxyl group, and two vicinal diols in the hydrocarbon chain (Table 1). The presence of one chloro atom was evidenced by peaks at *δ* 4.10 (1H) in the ^{1}H-NMR and at *δ* 60.4 in the ^{13}C-NMR spectrum. The proton resonance at *δ* 4.10, which was correlated with two peaks at *δ* 2.76 (H-3a) and *δ* 2.64 (H-3b) in the COSY spectrum, suggested that the chloro group was located at the C-4 position.

As has been shown by Downie *et al.*[11] and by Ried and Appel[12] phosphines attack CCl_4 at chlorine, forming a chlorophosphonium trichloromethide salt, which reacts with alcohols with inversion of the stereochemistry of the alcohol, in accord with a modified S_N2 reaction. The absolute stereochemistry of C-4 in gigantetrocin A has been determined as *R* by Mosher ester methodology, therefore, this position is most likely to be *S* in compound **1**. The other chiral centers in **1** are expected to retain their stereochemistries. Compound **1** was, thus, identified as 4(*S*)-chloro-4-deoxygigantetrocin A.

Compound **2** was isolated, from HPLC separation of the reaction mixture, as another colorless wax. CIMS gave three molecular ion peaks at *m*/*z* 614, 616, and 618, with the ratio of 9:6:1, indicating the presence of two chloro atoms. These were also evidenced by peaks at *δ* 4.04 and 4.14 in the ^{1}H-NMR and at *δ* 60.4 and 66.0 in the ^{13}C-NMR spectrum (Table 1). Again, the proton resonance at *δ* 4.10 was correlated with two peaks at *δ* 2.75 (H-3a) and *δ* 2.63 (H-3b) in the COSY spectrum, suggesting that one chloro group was located at the C-4 position. The proton resonance at *δ* 4.04 was correlated with a peak at *δ* 3.93, which was further correlated with two peaks at *δ* 1.98 (H-16a) and 1.75 (H-16b), suggesting that the second chloro atom was located adjacent to a THF ring. The molecular formula of **2**, $C_{35}H_{60}O_4Cl_2$, having five indices of hydrogen deficiency, indicated that a *bis*-THF ring had been formed. This was further evidenced by the ^{1}H signals at *δ* 3.93 (m, 4H, H-10, 13, 14, 17) and ^{13}C-NMR signals at *δ* 79.8 (C-10), 82.2 (C-13), 82.3 (C-14), and 80.4 (C-17). As with **1**, the stereochemistry of C-4 is proposed as *S*, and those at C-10 and C-13 are expected to be the same as those in gigantetrocin A. However, the absolute stereochemistries of the remaining chiral centers are difficult to predict, because the cyclodehydration of the 1,2,5-triol to form a THF ring could undergo different pathways, either by dehydration of the C-17,18 diol to form an epoxide followed by the attack of C-14 OH to open the epoxide or by cyclodehydration of the C-14,17 diol directly to form a THF ring. The two pathways would give different stereochemistries at C-14,17,18. Further mechanistic studies are needed to elucidate the stereochemical changes involved in the reaction. Thus, the structure of **2** was determined as illustrated and named as 4(*S*),18-dichloro-4,18-dideoxyasimilobin, although the stereochemistry may be different from that of asimilobin.[13]

Compounds **1** and **2** are moderately toxic to the brine shrimp larvae, and they show decreased bioactivities against the six human tumor cell lines as compared with the unchlorinated parent compounds, gigantetrocin A and asimilobin (Table 2). These results suggest that chlorination does not enhance, but decreases, the bioactivities of the annonaceous acetogenins. However, **1** was selectively cytotoxic to the colon cell line (HT-29),[14] and **2** was selectively cytotoxic to the prostate cell line (PC-3);[17] the selective potencies of both were about one-tenth that of Adriamycin.

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[⊗] Abstract published in *Advance ACS Abstracts,* October 1, 1996.

S0163-3864(96)00508-3 CCC: $12.00

Table 1. ^{1}H and ^{13}C-NMR Data of Compounds **1** and **2**

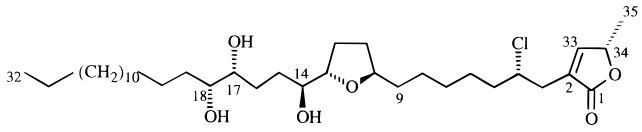
	1		2	
H/C no.	^{1}H	^{13}C	^{1}H	^{13}C
1		174.1		174.2
2		130.0		130.0
3a	2.76 ddt (15.1, 3.2, 1.6)	22.7−37.7	2.75 ddt (15.1, 3.2, 1.6)	22.7−37.7
3b	2.64 ddt (15.1, 8.2, 1.3)		2.63 ddt (15.1, 8.2, 1.3)	
4	4.15 m	60.4	4.14 m	60.4
5−9	1.20−1.50 m	22.7−37.7	1.20−1.50 m	22.7−37.7
10	3.89 m	79.2	3.93 m	79.8
11a, 12a	1.98 m	22.7−37.7	1.98 m	22.7−37.7
11b, 12b	1.53−1.70 m	22.7−37.7	1.75 m	22.7−37.7
13	3.81 m	81.8	3.93 m	82.3[b]
14	3.45 m	74.3	3.93 m	82.2[b]
15a, 16a	1.20−1.50 m	22.7−37.7	1.98 m	22.7−37.7
15b, 16b	1.20−1.50 m	22.7−37.7	1.53−1.70 m	22.7−37.7
17	3.45 m	74.4	3.93 m	80.4
18	3.45 m	74.3	4.04 m	66.0
19−31	1.20−1.50 m	22.7−37.7	1.20−1.50 m	22.7−37.7
32	0.88 t (6.9)	14.1	0.88 t (6.9)	14.0
33	7.19 q (1.5)	152.0	7.19 q (1.5)	152.2
34	5.06 qq (6.5, 1.5)	77.7	5.06 qq (6.5, 1.5)	77.7
35	1.43 d (6.5)	19.0	1.43 d (6.5)	19.0

[b] Signals may be interchangeable.

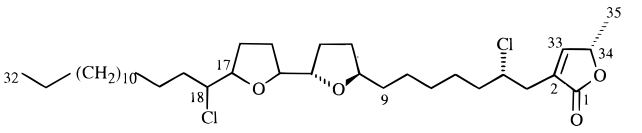
Table 2. Bioactivity Data of Compounds **1** and **2** (LC_{50} and ED_{50}: μg/mL)

compound	BST[a]	A-549[b]	MCF-7[c]	HT-29[d]	A-498[e]	PC-3[f]	PaCa-2[g]
1	54.6	>10	5.74	3.8×10^{-1}	>10	>10	>10
2	31.9	2.39	>10	>10	2.47	7.55×10^{-1}	1.16
gigantetrocin A	2.6	2.5×10^{-1}	6.3×10^{-1}	4.1×10^{-5}	NT	NT	NT
asimilobin	1.06	3.01×10^{-2}	2.14	6.3×10^{-2}	2.26×10^{-2}	1.47	1.04×10^{-1}
adriamycin	NT	2.43×10^{-2}	2.09×10^{-1}	3.46×10^{-2}	6.49×10^{-3}	2.62×10^{-2}	7.90×10^{-3}

[a] Brine shrimp lethality test.[6,7] [b] Human lung carcinoma.[15] [c] Human breast carcinoma.[16] [d] Human colon adenocarcinoma.[14] [e] Human kidney carcinoma.[15] [f] Human prostate adenocarcinoma.[17] [g] Human pancreatic carcinoma.[18]



1



2

Experimental Section

General Experimental Procedures. LRMS were recorded on a Finnigan 4000 mass spectrometer. The exact masses were determined on a Kratos MS 50 mass spectrometer through peak matching. ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian VXR-500S spectrometer, using the Varian software systems. HPLC was carried out with a Rainin HPLC instrument using the Dynamax software system and a Si gel column (250 × 21 mm) equipped with a Rainin UV-1 detector set at 220 nm.

Bioassays. The BST was conducted in our laboratory.[6,7] The cytotoxicity tests against A-549 (human lung carcinoma),[15] MCF-7 (human breast carcinoma),[16] HT-29 (human colon adenocarcinoma),[14] A-498 (human kidney carcinoma),[15] PC-3 (human prostate adenocarcinoma),[17] and PaCa-2 (human pancreatic carcinoma)[18] cells were performed in the Purdue Cell Culture Laboratory, Purdue Cancer Center, using standard protocols in 7-day assays using MTT with Adriamycin as a positive standard control.

Preparation of Chlorinated Acetogenins. Gigantetrocin A (50 mg, 0.08 mmol) and 45 mg (0.17 mmol) of TPP were dissolved in 1 mL of CCl_4. After refluxing for 16 h, the mixture was purified over a micro-column (0.6 × 6 cm) of Si gel eluted with 2 mL of CH_2Cl_2. The eluate was purified by HPLC (Si gel column, 10% MeOH−THF, 9:1 in hexane) to give compounds **1** and **2**.

4(*S*)-Chloro-4-deoxygigantetrocin A (1): colorless wax; yield (7.3%); mp 65 °C; $[\alpha]_D$ +6.1° (*c* 0.1, $CHCl_3$); UV λ max (ϵ) (MeOH) 219 nm (8500); IR, (film) υ max 3433, 2920, 1745, 1321 cm^{-1}; CIMS *m/z* 614, 616; ^{1}H and ^{13}C NMR See Table 1.

4(*S*),18-Dichloro-4,18-dideoxyasimilobin (2): colorless wax; yield (32.6%); mp 58 °C; $[\alpha]_D$ +16.1° (*c* 0.1, $CHCl_3$); UV λ max (ϵ) (MeOH) 217 nm (8500); IR, (film) υ max 2921, 1745, 1321 cm^{-1}; CIMS *m/z* 614, 616, 618; ^{1}H and ^{13}C NMR See Table 1.

Acknowledgment. This work was supported by Grant no. RO1 CA30909 from the National Cancer Institute, National Institute of Health. Cytotoxicity data were provided by the Cell Culture Laboratory, Purdue University Cancer Center.

References and Notes

(1) Rupprecht, J. K.; Hui, Y. H.; McLaughlin, J. L. *J. Nat. Prod.* **1990**, *53*, 237−278.
(2) Fang, X. P.; Rieser, R. J.; Gu, Z. M.; Zhao, G. X.; McLaughlin, J. L. *Phytochem. Anal.* **1993**, *4*, 27−67.
(3) Gu, Z. M.; Zhao, G. X.; Oberlies, N. H.; Zeng, L.; McLaughlin, J. L. *Recent Advances in Phytochemistry*; Arnason, J. T., Mata, R., Romeo, J. T., Eds. Plenum Press: New York, 1995; Vol. 29, pp 249−310.
(4) Zeng, L.; Ye, Q.; Oberlies. H. N.; Shi, G.; Gu, Z. M.; He, K.; McLaughlin, J. L. *Nat. Prod. Rep.* **1996**, *13*, 275−306.

(5) Cavé, A. In *Phytochemistry of Plants Used in Traditional Medicine*, Hostettmann, K., Marston, A., Maillard, M., Hamburger, M., Eds.; Clarendon Press: Oxford, 1995; pp 227−248.
(6) Meyer, B. N.; Ferrigni, N. R.; Putnam, J. E.; Jacobson, L. B.; Nichols, D. E.; McLaughlin, J. L. *Planta Med.* **1982**, *45*, 31−34.
(7) McLaughlin, J. L. In *Methods in Plant Biochemistry*; Hostettmann, K., Ed.; Academic Press: London, 1991; Vol. 6, pp 1−32.
(8) Barry, C. N.; Evans, S. A., Jr. *Tetrahedron Lett.* **1983**, 661−664.
(9) Fang, X.-P.; Rupprecht, J. K.; Alkofahi, A.; Hui, Y.-H.; Liu, Y.-M.; Smith, D. L.; Wood, K. V.; McLaughlin, J. L. *Heterocycles* **1991**, *32*, 11−17.
(10) Rieser, M. J.; Fang, X.-P.; Anderson, J. E.; Miesbauer, L. R.; Smith, D. L.; McLaughlin, J. L. *Helv. Chim. Acta* **1993**, *76*, 2433−2444; **1994**, *77*, 882 (Erratum).
(11) Downie, I. M.; Lee, J. B.; Matough, M. F. S. *Helv. Chim. Acta* **1969**, 1017−1021.
(12) Ried, W.; Appel, H. *Justus Liebigs Ann. Chem.* **1964**, *679*, 51−57.
(13) Woo, M. H.; Cho, K.-Y.; Zhang, Y.; Zeng, L.; Gu, Z.-M.; McLaughlin, J. L. *J. Nat. Prod.* **1995**, *58*, 1533−1542.
(14) Fogh, J.; Trempe, G. in *Human Tumor Cell Lines* In Vitro; Fogh, J., Ed.; Plenum Press: New York, 1972; pp 115−159.
(15) Giard, D. J.; Aronson, S. A.; Todaro, G. J.; Arnstein, P.; Kersey, H. J.; Dosik, H.; Parks, W. P. *J. Natl. Cancer Inst.* **1973**, *51*, 1417−1423.
(16) Soule, H. D.; Vazquez, J.; Long, A.; Albert, S.; Brennan, M. *J. Natl. Cancer Inst.* **1973**, *51*, 1409−1413.
(17) Kaighn, M. E.; Narayan, K. S.; Ohinuki, Y.; Lechner, J. F.; Jones, L. W. *Invest. Urol.* **1979**, *17*, 16−23.
(18) Yunis, A. A.; Arimura, G. K.; Russian, D. *Int. J. Cancer* **1977**, *19*, 128−135.

NP9605089