ANNORETICUIN AND ISOANNORETICUIN: TWO NEW CYTOTOXIC ACETOGENINS FROM *ANNONA RETICULATA*

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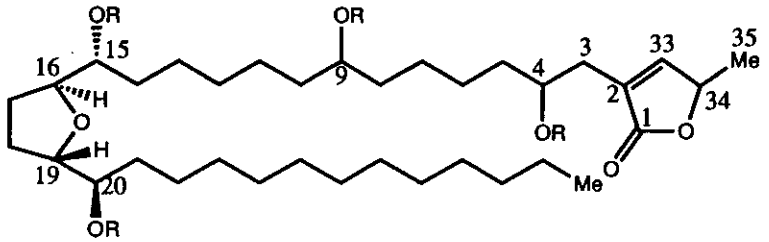
Abstract- Two novel bioactive monotetrahydrofuran acetogenins, annoreticuin (1) and isoannoreticuin (2), have been isolated from the leaves of Formosan plant *Annona reticulata* L. (Annonaceae). The plane structures of these two new compounds were elucidated on the basis of ^{1}H and ^{13}C nmr data and by mass spectrometry. These two compounds showed selective cytotoxic activities for certain human tumor cell lines.

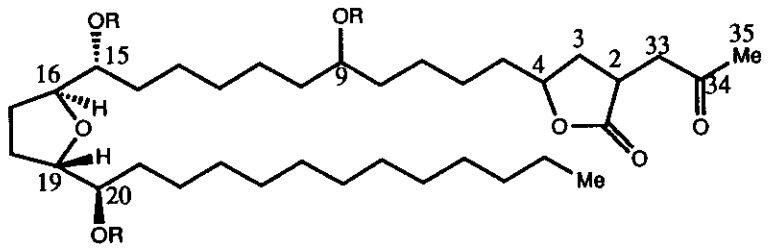
Annona reticulata L., commonly called "Niou Shin Li" in Formosan,[1] is a popular tropical fruit and has the common names of custard apple or bullocks heart in the world.[2] Over the past ten years, investigations of several genera of the family Annonaceae have resulted in the characterization of 48 biologically active acetogenins containing one or two tetrahydrofuran (THF) rings, a terminal r-lactone, and several hydroxyl moieties.[3-12] Almost all the compounds of both the C_{35} and C_{37} series have been found to possess potential cytotoxic, antitumor or pesticidal activities and, therefore,

these compounds have attracted much interest. To the best of our knowledge, previous phytochemical studies of A. reticulata have resulted in the isolation of a number of alkaloids,[13] a series of diterpenes,[14-16] and several bioactive acetogenins, 14-hydroxy-25-deoxyrollinicin,[3,17] reticulatacin,[8] annonareticin[18] and bullatacin.[19] As part of our continuing investigation of the phytochemical and bioactive agents of the Annonaceae, the MeOH extract of the leaves of Formosan plant A. reticulata was found to show significant cytotoxicity against in vitro tissue culture cells in human KB, A-549 lung carcinoma and HCT-8 colon tumor, as well as in murine P-388 and L-1210 lymphocytic leukemia. In this communication we report the bioactivity-directed isolation, structural elucidation and biological activities of two novel monotetrahydrofuran acetogenins, which we named annoreticuin (1) and isoannoreticuin (2).

Annoreticuin (1) was isolated as a white amorphous powder, $[\alpha]_D^{24}$ = +10.5° (c 0.02, $CHCl_3$). The high resolution chemical ionization (isobutane) mass spectra (HRCIms) gave m/z 597.4685 (calcd 597.4730) for the MH$^+$ corresponding to the molecular formula, $C_{36}H_{64}O_7$. The presence of four hydroxyl moieties was suggested by four successive losses of water (m/z 18) from the molecular ion in the CIms. In addition, the ir spectrum contained a broad absorption band at 3450 cm^{-1}, consistent with the presence of hydroxyl groups. The existence of four hydroxyl groups was confirmed by the preparation of a tetratrimethylsilyl derivative (3) [TMS, bis(trimethylsilyl)acetamide in pyridine] as evidenced by MH$^+$ ion corresponding to [MH + 4 TMS]$^+$ in the CIms. A prominent ir carbonyl absorption at 1745 cm^{-1} and a uv λ max at 210 nm (ε =4.01x10^3) suggested the presence of the α,β-unsaturated r-lactone. Expected resonances in the ^1H nmr and ^{13}C nmr (Table 1) confirmed the presence of an α,β-unsaturated r-lactone as well as the presence of a hydroxyl group at C-4.[3] Subsequent ^1H-^1H decoupling experiments showed coupling between the protons on C-3, H-3a and H-3b, to the single proton at C-4, establishing the presence of a hydroxyl group at C-4 as in annonacin,[20]



1 R=H
3 R=TMS
4 R=Ac



2 R=H
5 R=TMS

Table 1. Nmr data (ppm, Hz) for Annoreticuin (1) and Isoannoreticuin (2)

	Annoreticuin		Isoannoreticuin	
No. of Corbon	^1H 200 MHz $CDCl_3$	^{13}C 50 MHz $CDCl_3$	^1H 200 MHz $CDCl_3$	^{13}C 50 MHz $CDCl_3$
1		175.38 s		180.37 s
2		131.40 s	3.03 ddd (17,10,3.2)	34.76 d
3a	2.40 dd (14, 7.5)	37.41 t	2.23 m	33.75 t
3b	2.55 dd (14, 3.2)		1.96 m	
4	3.82 m	70.08 d	4.53 m	79.53 d
5-8	1.26-1.60 m	22-38 t	1.20-1.60 m	22-38 t
9	3.60 m	72.01 d	3.58 m	72.27 d
10-14	1.26-1.60 m	22-38 t	1.20-1.60 m	22-38 t
15,20	3.41 m	74.29 d 74.85 d	3.41 m	74.69 d 74.73 d
16,19	3.79 m	83.40 d 83.55 d	3.79 m	83.39 d 83.44 d
17,18	1.67,1.98 m	22-38 t	1.67,1.99 m	22-38 t
21-31	1.26-1.60 m	22-38 t	1.20-1.60 m	22-38 t
32	0.88 t(6.8)	14.27 q	0.88 t (6.8)	14.24 q
33	7.20 d(1.4)	152.64 d	a 2.68 dd (17, 9.5) b 2.98 dd (9.5, 3.2)	44.62 t
34	5.07 qd (7.0, 1.4)	79.89 d		207.36 s
35	1.44 d(7.0)	19.23 q	2.20 s	29.61 q

J(in Hz) in parentheses

and goniothalamicin.[21]

In addition to the resonances due to the oxygenated carbons of the lactone

and the four hydroxylated carbons at δ 74.85, 74.29, 72.01 and 70.08, the ^{13}C nmr showed two resonances at δ 83.55 and 83.40 also due to oxygen bearing carbons. These ^{13}C nmr resonances and their corresponding ^{1}H nmr resonances at δ 3.79 were directly analogous to similar signals of the non-adjacent rings in annonacin[20] and goniothalamicin,[21] indicating the presence of a single tetrahydrofuran moiety.

The placememt of the hydroxyl groups alpha to the tetrahydrofuran functionality was established by ^{1}H-^{1}H decoupling experiments that linked the two-proton signal at δ 3.41 to another two proton signal at δ 3.79. The subsequent downfield shift of these signals to δ 3.98(m) in the ^{1}H nmr of the aeetate derivative (4) confirmed this assignment. The single remaining hydroxyl group gave a one-proton signal at δ 3.60 (m) in the ^{1}H nmr; irradiation of this resonance indicated that this hydroxyl group was isolated along the alkyl chain. To determine the placement of the tetrahydrofuran ring and the isolated hydroxyl group along the hydrocarbon chain, mass spectral studies were undertaken.

A comparison of the EIms of 1 and 3 (Figure 1) established the carbon skeleton and the hydroxylation pattern for 1. Fragment in the EIms of 3 at m/z 613 and corresponding signals in the EIms of 1 clearly positioned the tetrahydrofuran ring at C-16 along the hydrocarbon chain and allowed the

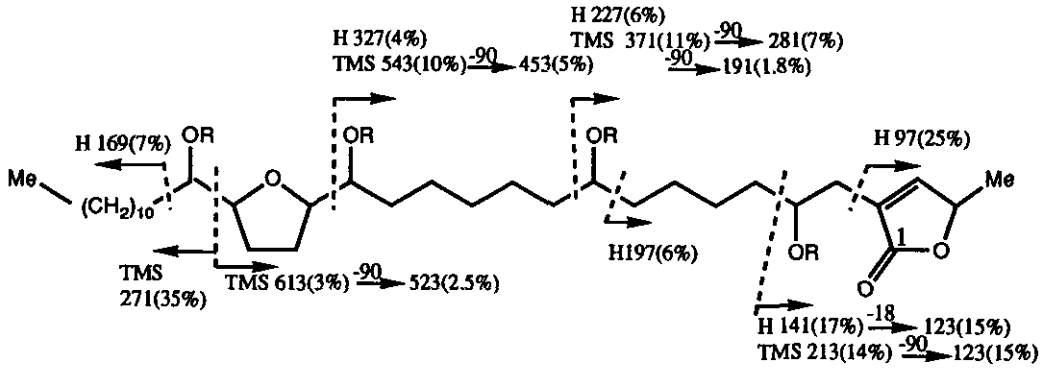
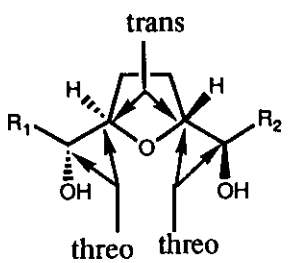
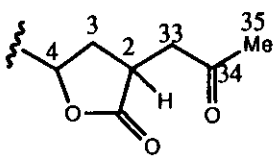


Figure 1. Diagnostic EIms fragment ions (m/z) of annoreticuin (1). "R" designates : the underivatized material (H) and the trimethylsilyl derivative (TMS); the ions of m/z -18 and m/z -90 are indicative of losses of H_2O and TMSOH, respectively.

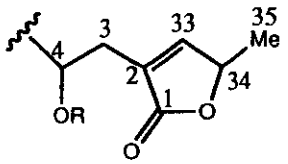
assignment of the hydroxyl groups at C-15 and C-20 adjacent to the tetrahydrofuran ring. The position of the remaining isolated hydroxyl moiety at C-9 was illustrated by fragments in the EIms of 1 and 3 at m/z 227 and 371, respectively. This fragment showed two consecutive losses of TMSOH to give at m/z 281 and 191. This fragmentation pattern was supported by the analogus fragments of 1. The FABms [MH$^+$ 597(100%) $\xrightarrow{-H_2O}$ 579(12%) $\xrightarrow{-H_2O}$ 561(10%) $\xrightarrow{-H_2O}$ 543(12%) $\xrightarrow{-H_2O}$ 525(22%)] confirmed the exact mass and the number of hydroxyl moieties of 1.

The relative stereochemistry of the tetrahydrofuran ring and the two adjacent hydroxyl groups at C-15 and C-20 was deduced by close examination of the nmr data. In the spectra of annoreticuin (1), the ^{13}C nmr chemical shifts of C-15, C-16, C-19 and C-20 at δ 74.29, 83.40, 83.55 and 74.85 clearly showed a threo relationship between C-15/C-16 and a threo relationship between C-19/C-20.[3] These assignments are based on the ^{13}C nmr chemical shifts of a pair of model monotetrahydrofuran compounds with adjacent hydroxyls of the threo and erythro configuration.[3] In annoreticuin tetraacetate (4), the ^{1}H nmr chemical shifts of H-16 and H-19 at δ 3.98 indicated the trans relationship for H-16/H-19.[3] Thus, the relative configuration of threo, trans , threo from C-15 to C-20 is evident (Figure 2).





A



B

Figure 2. Relative configuration of 1 and 2

Isoannoreticuin (2) was separated as a white amorphous powder, $[\alpha]_D^{24}$= +9.7° (c 0.33, $CHCl_3$). The molecular weight of 596 was established by CIms (Methane): 597 (MH$^+$)[HRCIms (M + H$^+$, 597.4741, calcd for $C_{36}H_{65}O_7$, 597.4730)] and based on this 2 was isomeric with 1, $C_{35}H_{64}O_7$. The ir spectrum showed

absorptions at 1700 cm^{-1} for a ketone and 1760 cm^{-1} for a r-lactone. This lactone was not α,β-unsaturated as in 1 since the compound was transparent in the uv at 190 nm. The signals for the butenolide ring were also missing in the ^{13}C and ^{1}H nmr of 2 (Table 1). Signals at δ 4.53 (m), consistent with a proton on a carbon attached to a lactone oxygen, and δ 2.20 (s), assigned to a methyl group adjacent to a ketone, suggested partial structure (A), which is isomeric with the butenolide structure (B) in the normal series. This isomeric relationship was confirmed by conversion of 1 to 2 by base-catalyzed isomerization.[22] The major product from treatment of 1 with KOH in tert-butyl alcohol was identical with 2 by comparison of ir, ms, and nmr data. The location of the hydroxyl groups was also determined on the basis of mass spectral fragmentation patterns and the key fragments of the EIms of 2 and 5 (Figure 3). From the above data, we concluded that the structures of annoreticuin and isoannoreticuin are as illustrated for 1 and 2, respectively, with the absolute and certain relative stereochemistry remaining undefined.

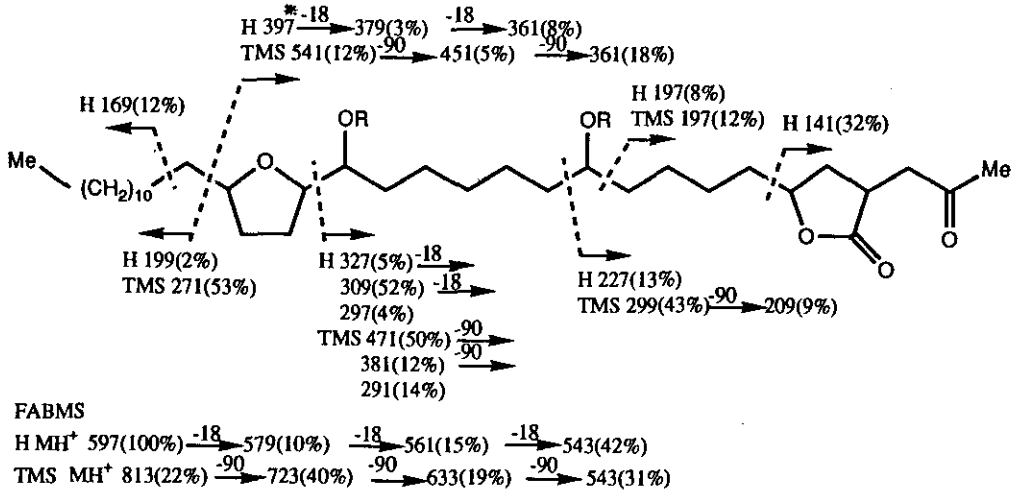


Figure 3. Diagnostic EIms fragment ions (m/z) of isoannoreticuin (2). "R" designates : the underivatized material (H) and the trimethylsilyl derivative (TMS) ; the ions of m/z -18 and m/z -90 are indicative of losses of H_2O and TMSOH, respectively. Asterisks* indicate that peaks cannot be seen, but the corresponding peaks formed by consecutive loss of one or two molecules of water were evident.

These two new acetogenins, annoreticuin (1) and isoannoreticuin (2), exhibited significant cytotoxicity in A-549 (human lung carcinoma), HT-29 (human colon adenocarcinoma), KB (human nasophryngeal carcinoma), and P-388 (mouse lymphocytic leukemia) cell culture systems. The cytotoxic activities of these two compounds are shown in Table 2.

Table 2. Cytotoxicity of 1 and 2

compound	ED_{50} μg/ml			
	P-388	A-549	KB	HT-29
1	3.60	0.43	3.37	2.28
2	1.02	0.41	6.69	3.06

ACKNOWLEDGEMENT

This investigation was supported by grant NSC-80-0208-M037-08 from National Science Council of R.O.C. awared to Y. C. Wu.

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Received, 6th January, 1992