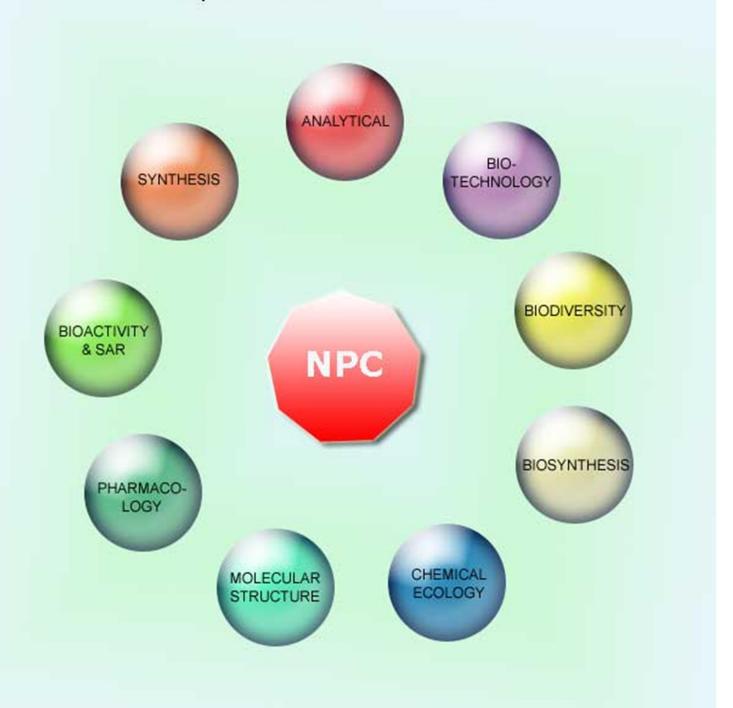
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ent-Kaurane Diterpenes from Annona glabra and Their Cytotoxic Activities

Hoang Le Tuan Anh^a, Nguyen Thi Thu Hien^{a,c}, Dan Thi Thuy Hang^a, Tran Minh Ha^a, Nguyen Xuan Nhiem^a, Truong Thi Thu Hien^b, Vu Kim Thu^c, Do Thi Thao^d, Chau Van Minh^a and Phan Van Kiem^{a,*}

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A new *ent*-kaurane glycoside, annoglabasin H (1), and three known *ent*-kauranes, annoglabasin E (2), annoglabasin B (3), and 19-nor-*ent*-kaurent-4-ol-17-oic acid (4) were isolated from the fruits of *Annona glabra*. Their structures were determined by the combination of spectroscopic and chemical methods, including 1D- and 2D-NMR spectroscopy, as well as by comparison with the NMR data reported in the literature. The cytotoxic activities of these compounds were evaluated on four human cancer cell lines, LU-1, MCF-7, SK-Mel2, and KB. Compound 1 exhibited significant cytotoxic activity on all tested human cancer cell lines with IC_{50} values ranging from 3.7 to 4.6 μ M.

Keywords: Annona glabra, Annonaceae, Annoglabasin H, Cytotoxic activity.

Annona glabra L., family Annonaceae, is a tropical tree growing wild in the Americas and Asia. It is used in traditional medicine to treat several diseases such as inflammation and cancer, and as an insecticide. Phytochemical investigation led to the isolation of numerous acetogenins [1], ent-kauranes [2-5], peptides [6], and alkaloids [7-8]. In addition, compounds exhibited anticancer [5-9], anti HIV-reverse transcriptase [4], and anti-malarial activities [7]. As part of our continuing efforts to find new anticancer compounds, one new ent-kaurane glycoside and three known ent-kauranes were isolated from the fruits of A. glabra (Figure 1).

Compound 1 was isolated as a white amorphous powder and its molecular formula was determined to be C₃₂H₅₀O₁₄ by HR-ESI-MS from the ion at m/z 681.3095 (Calcd. for $C_{32}H_{50}O_{14}Na$: 681.3093). The ¹H NMR spectrum of 1 showed signals for two tertiary methyl groups at δ_H 0.97 (3H, s) and 1.24 (3H, s), assigned to an ent-kaurane structure; and two anomeric protons at $\delta_{\rm H}$ 5.43 (d, J = 8.0 Hz) and 5.53 (d, J = 8.0 Hz), which suggested the presence of two sugar moieties. The ¹³C NMR and DEPT spectra of 1 revealed signals for 32 carbons including two carbonyl, three quaternary, fourteen methine, eleven methylene, and two methyl (Table 1). The ¹H and ¹³C NMR data of 1 were very similar to those of 16α-hydro-ent-kauran-17,19-dioic acid except for the addition of two sugar moieties at C-17 and C-19 [10]. The HMBC correlations between H-18 (δ_H 1.24) and C-3 (δ_C 39.0)/C-4 (δ_C 45.1)/C-5 (δ_C 58.6)/C-19 (δ_C 178.4) suggested the presence of both methyl and carboxyl groups at C-4. The HMBC correlations from H-13 (δ_H 2.55)/H-15 (δ_H 1.59 and 1.87)/H-16 $(\delta_{\rm H}~3.06)$ to C-17 $(\delta_{\rm C}~175.3)$ confirmed the position of a carboxyl group at C-16. The HMBC correlations between H-1' (δ_H 5.53) and C-17 (δ_C 175.3); and H-1" (δ_C 5.43) and C-19 (δ_C 178.4) confirmed the positions of two glucopyranosyl moieties at C-17 and C-19. The observation of NOESY correlation between H-18 $(\delta_H \ 1.24)$ and H-5 $(\delta_H \ 1.15)$, but not between H-18 $(\delta_H \ 1.24)$ and H-20 (δ_H 0.97) (Figure 1) confirmed the β configuration of the

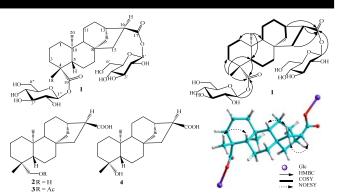


Figure 1: The chemical structures of 1-4 and important HMBC, COSY, and NOESY correlations of 1.

Table 1: ¹H (500 MHz) and ¹³C (125 MHz) NMR data for compound 1 in CD₃OD.

C	δ_{C}	$\delta_{\rm H}$ (mult., J in Hz)	C	$\delta_{\rm C}$	$\delta_{\rm H}$ (mult., J in Hz)
1	41.4	0.87 (m)/1.88 (m)	18	29.0	1.24 (s)
2	19.2	1.52 (m)/1.69 (m)	19	178.4	-
3	39.0	1.11 (m)/1.21 (d, 9.0)	20	16.4	0.97 (s)
4	45.1	-	17- <i>O</i> -Glc		
5	58.6	1.15 (m)	1'	95.6	5.53 (d, 8.0)
6	23.2	1.88 (m)/2.00 (m)	2'	74.0	3.35 (m)
7	42.9	1.57 (m)/1.96 (m)	3'	78.7	3.48 (m)
8	45.6	-	4'	71.1	3.42 (m)
9	57.6	1.08 (m)	5'	78.7	3.40 (m)
10	40.8	-	6'	62.4	3.71 (dd, 2.0, 11.5)
					3.84 (d, 11.5)
11	20.1	1.43 (m)/1.94 (m)	19- <i>O</i> -Glc		
12	28.0	1.47 (m)/1.71 (m)	1"	95.6	5.43 (d, 8.0)
13	41.1	2.55 (m)	2"	74.0	3.38 (m)
14	41.9	1.17 (m)/2.16 (d, 12.0)	3"	78.7	3.48 (m)
15	42.7	1.59 (m)/1.87 (m)	4"	71.1	3.42 (m)
16	46.6	3.06 (m)	5"	78.7	3.40 (m)
17	175.3	-	6"	62.3	3.71 (dd, 2.0, 11.5)
					3.84 (d, 11.5)

methyl group at C-4. Moreover, NOESY correlations of H-16 ($\delta_{\rm H}$ 3.06) and H-13 ($\delta_{\rm H}$ 2.55); H-16 ($\delta_{\rm H}$ 3.06) and H_a-15 ($\delta_{\rm H}$ 1.59); and H-9 ($\delta_{\rm H}$ 1.08) and H_{β}-15 ($\delta_{\rm H}$ 1.87) were observed confirming

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the α -configuration of H-16. Acid hydrolysis of **1** provided D-glucose (identified as its TMS derivative) [12]. In addition, the coupling constants of glc H-1'/glc H-2'; glc H-1"/glc H-2", J = 8.0 Hz indicated that these protons all had *axial* orientations. Consequently, compound **1** was elucidated to be 16α -hydro-*ent*-kauran-17,19-dioic acid 17,19-di-O- β -D-glucopyranoside ester, a new compound named annoglabasin H.

Annoglabasin E (2) [3], annoglabasin B (3) [4], and 19-nor-ent-kauran-4-ol-17-oic acid (4) [10] were identified on the basis of spectral data, which were in good agreement with those reported in the literature.

Cytotoxic activities of the compounds were evaluated by a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay on four human cancer cell lines (LU-1, MCF-7, SK-Mel2, and KB) [11]. Compound 1 exhibited significant cytotoxic activity on all tested human cancer cell lines with IC_{50} values ranging from 3.7 to 4.6 μ M.

Table 2: The effects of compounds 1 - 4 on the growth of human cancer cell lines.

Compound	IC ₅₀ (μM)				
Compound	LU-1	MCF-7	SK-Mel2	KB	
1	4.1±0.3	4.6±0.4	3.7±0.3	4.4±0.5	
2	>100	>100	>100	>100	
3	>100	>100	>100	>100	
4	>100	>100	>100	>100	
Ellipticine	3.5±0.2	3.7±0.1	3.4±0.4	4.0±0.2	

^{*}Ellipticine was used as a positive control, LU-1 (Human lung carcinoma), MCF-7 (Human breast carcinoma), SK-Mel-2 (Human melanoma), and KB (Human oral carcinoma).

Experimental

General: NMR, Bruker DRX 500 spectrometer; HR-ESI-MS, AGILENT 6550 iFunnel Q-TOF LC/MS system; Optical rotations, Jasco DIP-370 automatic polarimeter.

Plant material: The fruits of *A. glabra* were collected in Ho Chi Minh City, Vietnam during May 2013, and identified by Dr Bui Van Thanh, Institute of Ecology and Biological Resources, VAST. A voucher specimen (AG1605) was deposited at the Herbarium of Institute of Marine Biochemistry, VAST.

Extraction and isolation: The dried fruits of A. glabra (4.0 kg) were extracted with MeOH (3×5 L, 50°C) under sonication for 1 h to yield

300.0 g extract after evaporation of the solvent. This extract was suspended in H₂O (2.0 L) and successively partitioned with *n*-hexane, CHCl₃, and ethyl acetate (EtOAc) to obtain the *n*-hexane (AG1, 51.0 g), CHCl₃ (AG2, 190.5 g), EtOAc (AG3, 3.5 g), and H₂O (AG4, 54.0 g) extracts after removal of the solvents in vacuo. The AG2 extract was chromatographed on a silica gel column and eluted with a *n*-hexane–EtOAc gradient (100 : 1–1 : 1, v/v) to obtain 4 fractions, AG2A-AG2D. The AG2B fraction was chromatographed on a silica gel column eluting with n-hexane-EtOAc (4:1, v/v) to obtain 3 fractions, AG2B1-AG2B3. The AG2B1 fraction was further chromatographed on an YMC RP-18 column eluting with acetone-H2O (3 : 1, v/v) to yield 3 (217.0 mg). The AG2D fraction was chromatographed on a silica gel column eluting with *n*-hexane–acetone (2:1, v/v) to obtain 3 fractions, AG2D1-AG2D3. The AG2D1 fraction was chromatographed on an YMC RP-18 column eluting with acetone-water (5:1, v/v) to yield 2 (10.0 mg). The AG2D2 fraction was chromatographed on an YMC RP-18 column eluting with acetone-water (2.5 : 1, v/v) to yield 4 (9.0 mg). The water soluble fraction AG4 was chromatographed on a Diaion HP-20P column (Mitsubishi Chem. Ind. Co., Tokyo, Japan) eluting with water containing increasing concentrations of MeOH (0, 25, 50, 75, and 100% MeOH) to give 4 fractions, AG4A-AG4D. The AG4C fraction was chromatographed on a silica gel column eluting with CH₂Cl₂-MeOH (6: 1, v/v) to give 3 fractions, AG4C1-AG4C3. The AG4C1 fraction was chromatographed on an YMC RP-18 column eluting with MeOH- H_2O (1: 1.5, v/v) to yield 1 (3.0 mg).

Annoglabasin H (1)

A white amorphous powder.

 $[\alpha]_D^{25}$: -40 (c 0.1, MeOH).

¹H and ¹³C NMR (CD₃OD): Table 1.

HR-ESI-MS found *m/z* 681.3095 (Calcd. for C₃₂H₅₀O₁₄Na: 681.3093).

Acid hydrolysis: The hydrolysis method used is described in [12]; the sugar in **1** was identified as its TMS derivative.

Cytotoxic tests: The cytotoxic assay is described in [12].

Acknowledgment. This research was supported by the Vietnam Academy of Science and Technology (VAST 04.04/13-14).

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