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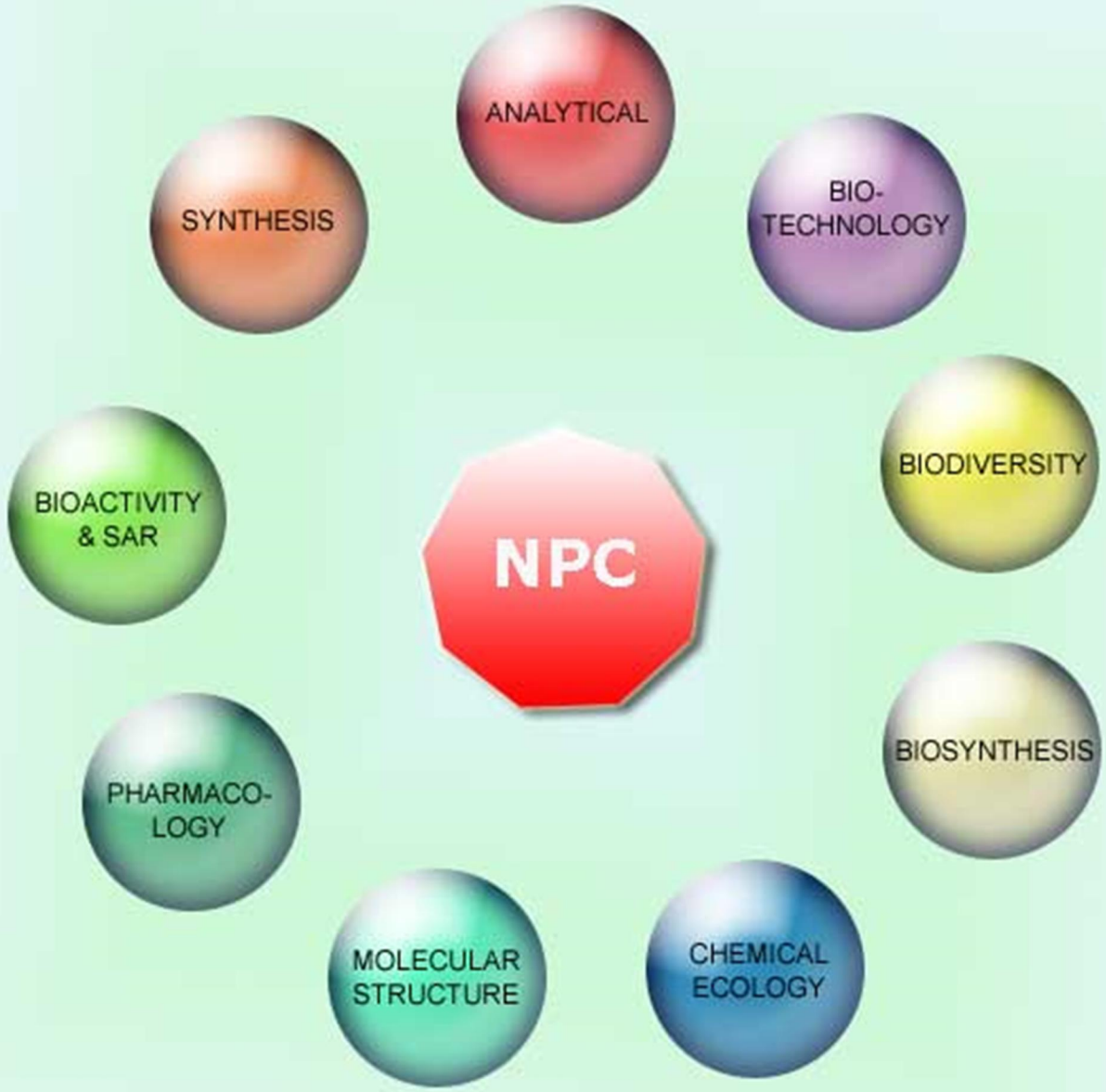

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ent-Kaurane Diterpenes from *Annona glabra* and Their Cytotoxic Activities

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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 μM.

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).

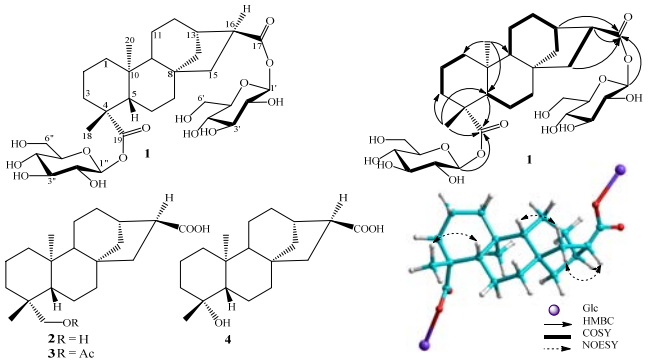

Figure 1: The chemical structures of **1**−**4** and important HMBC, COSY, and NOESY correlations of **1**.

Table 1: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data for compound **1** in CD_3OD.

C	δ_C	δ_H (mult., *J* in Hz)	C	δ_C	δ_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-*O*-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-*O*-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)

Compound **1** was isolated as a white amorphous powder and its molecular formula was determined to be $C_{32}H_{50}O_{14}$ by HR-ESI-MS from the ion at *m/z* 681.3095 (Calcd. for $C_{32}H_{50}O_{14}Na$: 681.3093). The ^{1}H 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 δ_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 ^{13}C 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 ^{1}H and ^{13}C NMR data of **1** were very similar to those of 16*α*-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 (δ_H 3.06) to C-17 (δ_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 (δ_H 1.24) and H-5 (δ_H 1.15), but not between H-18 (δ_H 1.24) and H-20 (δ_H 0.97) (Figure 1) confirmed the β configuration of the methyl group at C-4. Moreover, NOESY correlations of H-16 (δ_H 3.06) and H-13 (δ_H 2.55); H-16 (δ_H 3.06) and H_α-15 (δ_H 1.59); and H-9 (δ_H 1.08) and H_β-15 (δ_H 1.87) were observed confirming

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 μM.

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

Compound	IC_{50} (μM)			
	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 H_2O (2.0 L) and successively partitioned with *n*-hexane, $CHCl_3$, and ethyl acetate (EtOAc) to obtain the *n*-hexane (AG1, 51.0 g), $CHCl_3$ (AG2, 190.5 g), EtOAc (AG3, 3.5 g), and H_2O (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−H_2O (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 CH_2Cl_2−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 ^{13}C NMR (CD_3OD): Table 1.
HR-ESI-MS found *m/z* 681.3095 (Calcd. for $C_{32}H_{50}O_{14}Na$: 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].

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References

[1] Liu X-X, Alali FQ, Pilarinou E, McLaughlin JL. (**1999**) Two bioactive mono-tetrahydrofuran acetogenins, annoglacins A and B, from *Annona glabra*. *Phytochemistry*, ***50***, 815-821.
[2] Etse JT, Gray AI, Waterman PG. (**1987**) Chemistry in the Annonaceae, XXIV. Kaurane and kaur-16-ene diterpenes from the stem bark of *Annona reticulata*. *Journal of Natural Products*, ***50***, 979-983.
[3] Chen C-Y, Chang F-R, Cho C-P, Wu Y-C. (**2000**) *ent*-Kaurane diterpenoids from *Annona glabra*. *Journal of Natural Products*, ***63***, 1000-1003.
[4] Chang F-R, Yang P-Y, Lin J-Y, Lee K-H, Wu Y-C. (**1998**) Bioactive kaurane diterpenoids *from Annona glabra*. *Journal of Natural Products*, ***61***, 437-439.
[5] Chen CH, Hsieh TJ, Liu TZ, Chern CL, Hsieh PY, Chen CY. (**2004**) Annoglabayin, a novel dimeric kaurane diterpenoid, and apoptosis in Hep G2 cells of annomontacin from the fruits of *Annona glabra*. *Journal of Natural Products*, ***67***, 1942-1946.
[6] Li C-M, Tan N-H, Zheng H-L, Mu Q, Hao X-J, He Y-N, Zhou J. (**1999**) Cyclopeptides from the seeds of *Annona glabra*. *Phytochemistry*, ***50***, 1047-1052.
[7] Likhitwitayawuid K, Angerhofer CK, Cordell GA, Pezzuto JM, Ruangrungsi N. (**1993**) Cytotoxic and antimalarial bisbenzylisoquinolme alkaloids from *Stephania erecta*. *Journal of Natural Products*, ***56***, 30-38.
[8] Tsai S-F, Lee S-S. (**2010**) Characterization of acetylcholinesterase inhibitory constituents from *Annona glabra* assisted by HPLC microfractionation. *Journal of Natural Products*, ***73***, 1632-1635.
[9] Cochrane CB, Nair PKR, Melnik SJ, Resek AP, Ramachandran C. (**2008**) Anticancer effects of *Annona glabra* plant extracts in human leukemia cell lines. *Anticancer Research*, ***28***, 965-971.
[10] Hsieh T-J, Wu Y-C, Chen S-C, Huang C-S, Chen C-Y. (**2004**) Chemical constituents from *Annona glabra*. *Journal of the Chinese Chemical Society*, ***51***, 869-876.
[11] Alley MC, Scudiero DA, Monks A, Hursey ML, Czerwinski MJ, Fine DL, Abbott BJ, Mayo JG, Shoemaker RH, Boyd MR. (**1988**) Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. *Cancer Research*, ***48***, 589-601.
[12] Nhiem NX, Tung NH, Kiem PV, Minh CV, Ding Y, Hyun JH, Kang HK, Kim YH. (**2009**) Lupane triterpene glycosides from leaves of *Acanthopanax koreanum* and their cytotoxic activity. *Chemical and Pharmaceutical Bulletin*, ***57***, 986-989.

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