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A New Diterpenoid Dimer from *Annona glabra*

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Abstract: A new diterpenoid dimer annonebinide A has been isolated from the stems of *Annona glabra*. Its structure was determined to be *ent*-16α-hydroxykauran-17-yl *ent*-16β-kauran-17-oate on the basis of spectroscopic and chemical evidence.

Keywords: *Annona glabra*, diterpenoid dimer, *ent*-16α-hydroxykauran-17-yl *ent*-16β-kauran-17-oate.

Annona glabra L. (*Annonaceae*), commonly known as "pond apple", is a tropical tree distributed mainly in the American and in southeast Asia. It has been used as an insecticide and parasiticide[1-2]. The genus *Annona* comprises about 120 species, many of them grow in tropical area in America and a few grow in tropical area of Africa. Five species were planted in China. Many plant of the genus *Annona* are excellent fruits in tropical area, but they are used as medicine in antivirus and antitumor in folk medicine[3]. Therefore it is necessary to study further the chemistry and pharmacology of *Annona* plants in order to clarify the relationship between folk medicines and their ingredients. In our studies, a series of kaurane diterpenes and other compounds were isolated from the bark of *A. glabra*. Among them, a new diterpenoid dimer was first found in the family of *Annonaceae*, named annonebinide A (**1**). In this communication we report its structure.

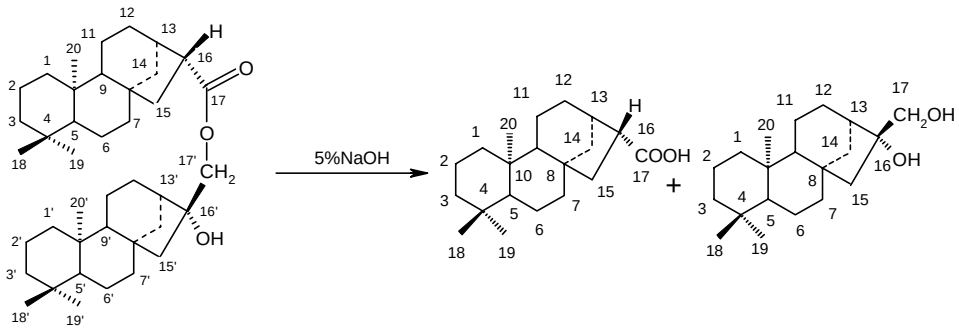
The air-dried stems (3.5 kg) of *A. glabra* were successively extracted with hot petroleum ether, EtOAc and EtOH. The EtOAc extract was fractionated by repeated silica gel column chromatography to yield annonebinide A.

Compound **1** was obtained as colorless needles (EtOAc), mp 275-277°C, $[\alpha]_D^{25}$-50 (c 0.25, $CHCl_3$), HRFAB-MS showed a molecular formula of $C_{40}H_{64}O_3$, *m/z* [M^+] 592.4768 (calcd. 592.4858). Its IR spectrum showed the presence of a hydroxyl group (3413cm^{-1}) and an ester carbonyl group (1703 cm^{-1}). The ^{1}H-NMR spectrum showed signals due to six tertiary methyl groups at δ 0.80 (s, 6H), 0.84 (s, 6H) , 0.99 (s, 3H) and 1.01 (s, 3H), one oxymethylene group at δ 4.02 (d, 1H, J=11.3Hz) and 3.86 (d, 1H, J=11.3Hz) (**Table 1**). The signals at chemical shifts of δ 0.80, 0.84 and 1.01 were similar to those of the tertiary methyl groups in the molecule of *ent*-kaurane-16α, 17-diol[4] isolated from *Fritillaria thunbergii* M, and the signals at chemical shifts of δ 0.84, 0.84, 0.99 were in

accord with those of the tertiary methyl groups in the molecule of *ent*-16β-hydroxy-kauran-17-oic acid[5]. The signals at chemical shifts of δ 4.02 and 3.86 belong to H-17′ of **1**, but the corresponding signal of **2** is at δ 3.49 and 3.37. The hydroxyl of C-16′ is tertiary and has stereoscopic obstruction, therefore, we concluded that the hydroxyl of C-17′ is esterified. The ^{13}C-NMR spectrum showed 40 carbon signals (**Table 2**) which were assigned to one ester carbonyl carbon at δ 177.8 and 39 carbon signals at up-field.

Comparison of the ^{1}H-NMR and ^{13}CNMR chemical shifts of **1** with those of **2** and **3**, suggested that **1** is a dimer derived from **2** and **3**, Alkaline hydrolysis of **1** yielded two compounds **2** and **3** (**Figure 1**).

Figure 1



1 **3** **2**

The structures of **2 and 3** were determined on the basis of spectroscopic and chemical evidence and by direct comparison with an authentic sample. Their spectral data were respectively identical with those reported in references (**Table 2**). The structure of **2**, $C_{20}H_{34}O_2$ (EI-MS *m/z* 306 M^+) was identified as *ent*-kaurane-16α, 17-diol[4]. The structure of **3**, $C_{20}H_{32}O_2$ (EI-MS *m/z* 304 M^+) was identified as *ent*-16β-hydroxy kauran-17-oic acid[5].

Table 1 ^{1}H-NMR spectral data for **1**, **2**, **3** (in $CDCl_3$, TMS as inner standard, 300MHz)

1 δ (ppm)		**2** δ (ppm)		**3** δ (ppm)
0.80 (s, 3H, 18-CH_3)		0.80 (s, 3H, 18-CH_3)		0.80 (s, 3H, 18-CH_3)
0.80 (s, 3H, 18′-CH_3)		0.84 (s, 3H, 20-CH_3)		0.85 (s, 3H, 20-CH_3)
0.84 (s, 3H, 20-CH_3)		1.03 (s, 3H, 19-CH_3)		1.00 (s, 3H, 19-CH_3)
0.84 (s, 3H, 20′-CH_3)				
0.99 (s, 3H, 19-CH_3)				
1.01 (s, 3H, 19′-CH_3)				
4.02 (d, 1H, J=11.3Hz)		3.49 (d, 1H, J=11.3Hz)		
	-C-CH_2-O-		-CH_2OH	
3.86 (d, 1H, J=11.3Hz)		3.37 (1 d, H, J=11.3Hz)		

Table 2 ^{13}C-NMR spectral data for **1**, **2**, **3,** and ent-kaurane-16α, 17diol, ent-16β-hydroxykauran-17-oic acid (300MHz, in $CDCl_3$, δ ppm)

C	**1**	**2**	**3**	*ent*-16β-hydroxykauran-17-oic acid	C	**1**	**2**	*ent*-kaurane-16α,17-diol	**3**
1	41.6		41.8	39.2	1′	41.8	41.9	42.0	
2	18.6		18.6	18.2	2′	18.6	18.7	18.2	
3	42.0		42.0	42.0	3′	42.0	42.0	42.0	
4	33.2		33.2	33.1	4′	33.2	33.2	33.4	
5	56.0		56.1	56.1	5′	56.2	56.1	56.1	
6	20.7		20.4	20.6	6′	20.0	20.0	20.5	
7	38.3		39.8	40.3	7′	38.2	38.2	37.2	
8	45.6		45.3	45.3	8′	43.7	43.5	44.6	
9	57.0		57.0	56.0	9′	57.0	56.9	56.7	
10	39.3		39.2	38.0	10′	39.2	39.2	39.4	
11	18.4		18.0	18.5	11′	18.4	18.6	18.3	
12	26.7		27.4	31.2	12′	26.8	26.7	26.3	
13	45.1		44.4	44.7	13′	52.6	52.6	45.5	
14	40.4		40.7	40.8	14′	40.4	40.4	40.4	
15	45.0		45.3	45.0	15′	56.0	56.1	53.4	
16	56.1		56.0	55.9	16′	78.8	79.7	81.6	
17	177.8		180.4	182.5	17′	71.0	69.1	66.2	
18	33.5		33.6	33.5	18′	33.6	33.6	33.4	
19	21.6		21.5	21.5	19′	21.6	21.5	21.5	
20	17.4		17.6	17.3	20′	17.5	17.6	17.7	

From the above evidence, the structure of **1** was established as *ent*-16α-hydroxy kauran-17-yl *ent*-16β-kauran-17-oate.

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