

## Two New Pentacyclic Triterpenes from *Abelmoschus esculentus*

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Two new pentacyclic triterpenes, (3 $\beta$ ,21 $\beta$ )-19,21-epoxylup-20(29)-en-3-yl acetate (**1**) and (3 $\beta$ )-9,18-dihydroxyolean-12-en-3-yl acetate (**2**), were isolated from *Abelmoschus esculentus* (L.) MOENCH. Their structures were determined on the basis of detailed analyses of their 1D- and 2D-NMR-spectroscopic and MS data.

**Introduction.** – *Abelmoschus esculentus* (L.) MOENCH (known as *okra*, *lady's fingers*) is considered to be of Indian origin. In India, its tender pods are used as a vegetable, and the seeds are used for making chutney, and its seeds as a substitute for coffee seeds [1]. In Egypt, corn flour is supplemented with flour of *Abelmoschus esculentus* seed in making bread to obtain a better quality dough [2]. Concerning the chemical composition of okra, different studies have been reported. K, Na, Mg, and Ca were found to be the principal elements, with Fe, Zn, Mn, and Ni being present as trace elements [3–5]. In our study on the chemical constituents of this plant, two new pentacyclic triterpenes, (3 $\beta$ ,21 $\beta$ )-19,21-epoxylup-20(29)-en-3-yl acetate (**1**) and (3 $\beta$ )-9,18-dihydroxyolean-12-en-3-yl acetate (**2**), were isolated (Fig. 1). Their structures were determined on the basis of detailed analyses of their 1D- and 2D-NMR-spectroscopic and MS data.

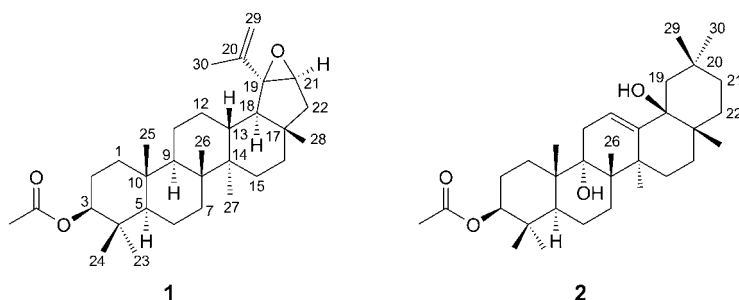


Fig. 1. Structures of compounds **1** and **2**

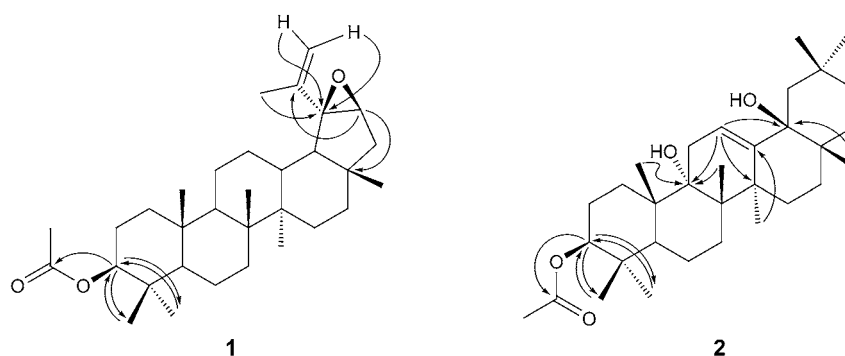
**Results and Discussion.** – Compound **1** was isolated as a white amorphous powder with a molecular formula  $C_{32}H_{50}O_3$ , deduced from the molecular-ion peak  $M^+$  at  $m/z$  482.3486 (calc. 482.3582) in the HR-ESI-MS. According to the IR spectrum, no OH group was present, but there were intensive bands for C–O stretching vibrations

around 1000 cm<sup>-1</sup>. The O-atom obviously forms an ether linkage. These results were consistent with the molecular formula C<sub>32</sub>H<sub>50</sub>O<sub>3</sub>, previously established on the basis of the <sup>13</sup>C-NMR and DEPT spectra. These spectra, together with the <sup>1</sup>H-NMR spectrum of **1** (Table 1) indicated the presence of seven tertiary Me groups ( $\delta$ (H) 0.87 (2 Me), 0.89 (2 Me), 0.98, 1.17, and 1.76), an acetyl Me group ( $\delta$ (H) 2.07), two vinyl H-atoms ( $\delta$ (H) 4.86 and 4.97), and two CH–O H-atoms ( $\delta$ (H) 4.51 and 4.06). These data allowed the identification of **1** as a pentacyclic triterpene of the lupane family by comparison of the <sup>13</sup>C-NMR chemical data with those of similar compounds [6]. The low-field Me signal at  $\delta$ (H) 1.76, and two vinyl H-atoms signals at  $\delta$ (H) 4.86 and 4.98 indicated the presence of isopropenyl group. The presence of an AcO–C(3) group was confirmed by a Me signal at  $\delta$ (H) 2.07 ( $\delta$ (C) 21.3) and a C=O signal group at  $\delta$ (C) 171.0. The presence of a H-atom at C(3) with a large coupling constant at ( $\delta$ (H) 4.51, ( $J = 10.2, 5.0$ )) evidenced the equatorial ( $\beta$ ) orientation of AcO. The H-atom with the signal at  $\delta$ (H) 4.06 was located at C(21) as evidenced from the HSQC and HMBC spectrum (Fig. 2). The <sup>13</sup>C-NMR (Table 1) spectrum of **1** exhibited 32 C-atom signals, indicating **1** to be a triterpene *O*-acetate. The low-field signals at  $\delta$ (C) 76.5 and 75.1 were attributed to C(21) and C(19), respectively, as evidenced from the HSQC and HMBC spectrum, the chemical shifts indicated the two C-atoms to be flanked by O-atoms. In the HMBC spectrum, the correlations from the Me(30) at  $\delta$ (H) 1.76, and two vinyl H-atoms at  $\delta$ (H) 4.86 and 4.98 to the quaternary C-atom at  $\delta$ (C) 75.07, due to C(19), were detected. The same spectra showed correlations from the H–C(21) at  $\delta$ (H) 4.06 to the quaternary C-atom at  $\delta$ (C) 110.9 (C(29)) and 36.6 (C(17)). Thus, from the above spectral data and the fragmentations observed in the HR-ESI-MS, the structure of compound **1** was determined as (3 $\beta$ ,21 $\beta$ )-19,21-epoxylup-20(29)-en-3-yl acetate (Fig. 1).

Table 1. Table 1. <sup>1</sup>H- and <sup>13</sup>C-NMR Data of Compound **1**.  $\delta$  in ppm,  $J$  in Hz.

Position	$\delta$ (H)	$\delta$ (C)	Position	$\delta$ (H)	$\delta$ (C)
1	1.65–1.72, 1.02–1.06 (2 <i>m</i> )	38.7	17	–	36.6
2	1.62–1.65 ( <i>m</i> )	23.7	18	1.34 ( <i>d</i> , $J = 5.9$ )	50.3
3	4.49 ( <i>dd</i> , $J = 10.0, 5.9$ )	80.9	19	–	75.1
4	–	37.9	20	–	147.7
5	0.81–0.85 ( <i>m</i> )	56.0	21	4.06 ( <i>t</i> , $J = 5.9$ )	76.5
6	1.48–1.52, 1.70–1.74 (2 <i>m</i> )	18.2	22	1.60–1.65, 1.24–1.28 (2 <i>m</i> )	29.3
7	1.50–1.53, 1.25–1.29 (2 <i>m</i> )	35.2	23	0.87 ( <i>s</i> )	28.0
8	–	40.4	24	0.87 ( <i>s</i> )	16.4
9	1.30–1.35 ( <i>m</i> )	50.6	25	0.89 ( <i>s</i> )	16.3
10	–	37.1	26	0.89 ( <i>s</i> )	16.5
11	1.22–1.26, 1.47–1.52 (2 <i>m</i> )	21.5	27	0.98 ( <i>s</i> )	15.5
12	1.70–1.75, 1.45–1.48 (2 <i>m</i> )	24.9	28	1.17 ( <i>s</i> )	25.4
13	1.58–1.62 ( <i>m</i> )	42.4	29	4.86, 4.97 (2 <i>s</i> )	110.9
14	–	31.2	30	1.76 ( <i>s</i> )	17.7
15	1.25–1.29, 1.80–1.85 (2 <i>m</i> )	27.5	AcO	2.07 ( <i>s</i> )	21.3, 171.0
16	1.70–1.76 ( <i>m</i> )	50.2			

Compound **2** was isolated as white amorphous powder, and its HR-ESI-MS showed the  $M^+$  peak at  $m/z$  500.3672 corresponding to the molecular formula C<sub>32</sub>H<sub>52</sub>O<sub>4</sub> (calc.

Fig. 2. Significant HMBCs of compounds **1** and **2**

500.7336). The NMR data indicate the presence of one C=C bond and one C=O group, suggesting five rings, most likely representing a triterpene with nine Me, ten CH<sub>2</sub>, three CH groups, and ten quaternary C-atoms (Table 2). The IR spectrum revealed the presence of OH groups (3510 cm<sup>-1</sup>) and an ester functionality (1732 cm<sup>-1</sup>). The <sup>1</sup>H-NMR spectrum (Table 2) exhibited signals for eight Me groups, all positioned at quaternary C-atoms ( $\delta(\text{H})$  0.87 (2 Me), 0.89 (2 Me), 0.98, 1.15, and 1.35 (2 Me)). The signal of an AcO Me group was observed at  $\delta(\text{H})$  2.07, corresponding to the C-atom signal at  $\delta(\text{C})$  21.3 in the HSQC experiment, with the AcO C=O group signal occurring at  $\delta(\text{C})$  171.0. In the HSQC experiment, the signal at  $\delta(\text{H})$  4.50 corresponded to the C-atom signal at  $\delta(\text{C})$  80.9 due to C(3). A H-atom signal at  $\delta(\text{H})$  5.72 corresponded to the only olefinic H-atom, and it correlated with the C-atom signal at  $\delta(\text{C})$  122.4 due to C(12) as indicated by HSQC. The signal of the other quaternary olefinic C-atom appeared at  $\delta(\text{C})$  142.0, and is due to C(13). The <sup>13</sup>C-NMR spectrum (Table 2) of **2**

Table 2. <sup>1</sup>H- and <sup>13</sup>C-NMR Data of Compound **2**.  $\delta$  in ppm,  $J$  in Hz.

Position	$\delta(\text{H})$	$\delta(\text{C})$	Position	$\delta(\text{H})$	$\delta(\text{C})$
1	1.65–1.72, 1.04–1.08 (2 <i>m</i> )	38.7	17	–	30.0
2	1.64–1.69 ( <i>m</i> )	23.7	18	–	70.8
3	4.48–4.53 ( <i>m</i> )	80.9	19	1.61–1.66, 1.72–1.76 (2 <i>m</i> )	42.4
4	–	37.9	20	–	29.9
5	0.81–0.85 ( <i>m</i> )	56.0	21	1.26–1.30, 1.50–1.55 (2 <i>m</i> )	35.1
6	1.48–1.53, 1.84–1.88 (2 <i>m</i> )	18.2	22	1.03–1.07, 1.44–1.48 (2 <i>m</i> )	31.1
7	1.70–1.76 ( <i>m</i> )	49.9	23	1.15 ( <i>s</i> )	25.7
8	–	40.4	24	0.98 ( <i>s</i> )	15.5
9	–	75.0	25	0.89 ( <i>s</i> )	16.3
10	–	38.7	26	0.89 ( <i>s</i> )	16.4
11	1.25–1.29, 1.47–1.52 (2 <i>m</i> )	23.7	27	1.35 ( <i>s</i> )	50.3
12	5.68–5.72 ( <i>m</i> )	122.4	28	1.35 ( <i>s</i> )	50.6
13	–	142.0	29	0.87 ( <i>s</i> )	27.5
14	–	43.5	30	0.87 ( <i>s</i> )	16.5
15	1.25–1.28, 1.82–1.86 (2 <i>m</i> )	27.9	AcO	2.07 ( <i>s</i> )	21.3, 171.0
16	1.58–1.64 ( <i>m</i> )	24.8			

showed 32 C-atom signals, indicating a triterpene *O*-acetate [7]. The low-field signals at  $\delta(\text{C})$  80.9, 75.0, and 70.8 were assigned to C(3), C(9), and C(18), respectively, as evidenced from the HSQC and HMBC spectrum; the chemical shifts indicated the C-atoms were O-bearing. The signals at  $\delta(\text{C})$  75.00 and 70.78 were attributed to quaternary C-atoms, based on the  $^{13}\text{C}$ -NMR and DEPT spectrum.

HSQC and HMBC (Fig. 2) spectra of **2** were utilized to assign the C- and H-atom signals. The C=C bond was established to be located between C(12) and C(13) due to HMBCs from Me(27) to C(13), and from H–C(12) to C(9), C(18), and C(14). The AcO group was assigned to be at C(3) based on the HMBCs observed from H–C(3) to the C=O C-atom. The correlations from H–C(3) to C(2), C(4), C(23), and C(24) could be detected. In addition, we observed a correlation of Me(3) to C(3). The OH groups were assigned to C(9) and C(18) based on the HMBC from H–C(12), Me(25), and Me(26) to C(9), and from Me(28) and H–C(12) to C(18). The configuration of the AcO group in **2**, was assigned as  $\beta$  due to the coupling constant ( $J = 10.3, 5.7$ ) of H–C(3) in the  $^1\text{H}$ -NMR. Thus, the structure of compound **2** was established as (3 $\beta$ )-9,18-dihydroxyolean-12-en-3-yl acetate (Fig. 1).

### Experimental Part

*General.* TLC: silica gel *GF*<sub>254</sub> (10–40  $\mu\text{m}$ ; Qingdao Marine Chemical Plant, Qingdao, P. R. China). Column chromatography (CC): silica gel ( $\text{SiO}_2$ , 200–300 mesh; Qingdao Marine Chemical Plant, Qingdao, P. R. China), *Sephadex LH-20* (GE Healthcare Bio-Sciences AB, USA), and *MCI* gel (*Mitsubishi Chemical Corporation*, Tokyo, Japan). IR Spectra (KBr): FT-IR PE-1710 spectrophotometer (*Perkin-Elmer*, USA);  $\tilde{\nu}$  in  $\text{cm}^{-1}$ .  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra: Bruker Avance DPX-400 spectrometer (*Bruker Corporation*, CH-Fällanden);  $\delta$  in ppm with  $\text{Me}_4\text{Si}$  as internal standard and  $J$  in Hz. HR-ESI-MS: Waters Q-TOF Micro mass spectrometer (*Waters Corporation*, Milford, USA); in  $m/z$ .

*Plant Materials.* The fruits of *Abelmoschus esculentus* (L.) MOENCH were collected in Jiangmen City of Guangdong Province, P. R. China, and identified by Prof. Chengxue Pan at the School of Pharmaceutical Sciences, Zhengzhou University. A voucher specimen is deposited with the School of Pharmaceutical Sciences, Zhengzhou University.

*Extraction and Isolation.* The fruits of *A. esculentus* (7.5 kg) were crushed and extracted with 95% EtOH under reflux. The extract was concentrated *in vacuo* to give a residue (351 g), which was dissolved in  $\text{H}_2\text{O}$ , and then partitioned with petroleum ether (42.4 g),  $\text{CHCl}_3$  (35.6 g), AcOEt (35 g), and BuOH (51 g), resp. The  $\text{CHCl}_3$  fraction was subjected to CC ( $\text{SiO}_2$ ; petroleum ether/AcOEt 100:0, 90:1, 40:1, 20:1, 5:1, 0:100) to yield seven fractions, *Frs. 1–7*. *Fr. 6* was purified by CC (*MCI* gel; MeOH) to give two subfractions. The *Subfr. 1* was submitted to CC ( $\text{SiO}_2$ ;  $\text{CHCl}_3$ /acetone 95:5) to give three subfractions, *Frs. 1A–1C*. *Fr. 1A* was separated by CC (*Sephadex LH-20*;  $\text{CHCl}_3$ /MeOH 2:1) to give **1**. The *Fr. 1B* was subjected to CC (*Sephadex LH-20*;  $\text{CHCl}_3$ /MeOH 2:1) to give **2**.

(3 $\beta$ ,21 $\beta$ )-19,21-Epoxyilup-20(29)-en-3-yl Acetate (**1**). White amorphous powder. IR (KBr): 1000, 885, 1640, 1735, 3070.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR: Table 1. HR-ESI-MS: 482.3486 ( $M^+$ ,  $\text{C}_{32}\text{H}_{50}\text{O}_3^+$ ; calc. 482.3582).

(3 $\beta$ )-9,18-Dihydroxyolean-12-en-3-yl Acetate (**2**). White amorphous powder. IR (KBr): 1647, 1732, 3510.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR: Table 2. HR-ESI-MS: 500.3672 ( $M^+$ ,  $\text{C}_{32}\text{H}_{52}\text{O}_4^+$ ; calc. 500.7336).

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