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New type sesquiterpene lactone from almond hulls (*Prunus amygdalus* Batsch)

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Abstract—A new unusual sesquiterpene lactone, named amygdalactone, was isolated from the hulls of almond (*Prunus amygdalus*). Complete assignment of the proton and carbon chemical shifts for the new lactone was accomplished on the basis of high-resolution 1D and 2D NMR data. Amygdalactone represents a new class of sesquiterpene with cyclohexa[7,12-*g*]octalactone ring system. The cytotoxic activity of amygdalactone was determined. © 2002 Elsevier Science Ltd. All rights reserved.

Almonds (*Prunus amygadus* Batsch) are one of the most popular tree nuts on a worldwide basis and rank number one in tree nut production. They belong to the Rosaceae family that also includes apples, pears, prunes, and raspberries.[1] Almonds are typically used as snack foods and as ingredients in a variety of processed foods, especially in bakery and confectionery products. The United States is the largest almond producer in the world and most of the US almonds are grown in California in an area that stretches over 400 miles from Bakersfield to Red Bluff.[2] Almond hulls, the outside jacket of almond meats, are mainly used as livestock feed. Previous studies by Takeoka et al.[3] had revealed that almond hulls are a rich source of triterpenoids, betulinic acid, oleanolic acid and ursolic acid, which have reported anti-inflammatory, anti-HIV, and anticancer activities. The very interesting pharmacological activities of these isolated triterpenoids prompted us to further investigate the chemical composition of almond hulls. In this report, we described the isolation and structure elucidation of a new type sesquiterpene lactone (**1**), named amygdalactone from the hulls of almond (*Prunus amygdalus* Batsch). Amygdalactone represents a new class of sesquiterpene with a cyclohexa[7,12-*g*]octalactone ring system.

The ethyl acetate fraction of almond hulls extract was chromatographed successively on silica gel, Sephadex LH-20 and prepared TLC plate to afford compound **1** (Fig. 1). Its structure was established by interpretation and full assignments of 1D and 2D NMR spectroscopic data.

Compound **1**[4] possesses the molecular formula $C_{15}H_{20}O_6$, as established according to the negative-ion APCI-MS ($[M-H]^-$ at m/z 295), as well as the ^{13}C NMR spectrum (Table 1). The ^1H and ^{13}C NMR spectral data showed that **1** had three singlet methyl groups (δ_H 1.11, H-15; 1.38, H-16; 2.03, H-13 and δ_C 14.7, C-15; 18.6, C-16; and 20.7, C-13, respectively). The most downfield methyl signal at δ_H 2.03 was assigned to the methyl attached to an olefinic double bond. The signals at δ_H 7.84, d, $J=16.2$, H-5; 6.26, d,

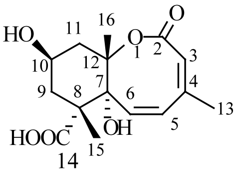

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Figure 1. Structure of compound **1**.

Keywords: almond hulls; *Prunus amygdalus*; Rosaceae; amygdalactone; sesquiterpene lactone.

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Table 1. NMR spectral data for compound **1** (CD_3OD) (δ in ppm, *J* in Hz)

	^1H	^{13}C	H–H COSY	HMBC
2		173.9 s		
3	5.91 s	127.2 d		C-2, C-5, C-13
4		143.0 s		
5	7.84 d, 16.2	134.4 d	H-6	C-3, C-4, C-7, C-13
6	6.26 d, 16.2	128.7 d	H-5	C-4, C-5, C-7
7		83.0 s		
8		53.6 s		
9a	1.77 dd, 9.6, 15.0	41.1 t	H-9e, H-10	C-7, C-8, C-10, C-14, C-15
9e	1.94 dd, 6.6, 15.0		H-9a, H-10	C-7, C-8, C-10, C-11
10	3.83 m	65.5 d	H-9e, H-9a, H-11e, H-11a	C-9, C-11
11a	1.89 dd, 9.6, 14.4	42.4 t	H-10, H-11e	C-7, C-10
11e	2.29 dd, 6.0, 14.4		H-10, H-11a	C-7, C-9, C-10, C-12, C-16
12		90.2 s		
13	2.03 s	20.7 q		C-3, C-4, C-5
14		181.5 s		
15	1.11 s	14.7 q		C-7, C-8, C-9, C-14
16	1.38 s	18.6 q		C-7, C-11, C-12

J = 16.2, H-6; 5.91, s, H-3; and δ_C 134.4 (d), C-5; 128.7 (d), C-6; 143.0 (s), C-4; and 127.2 (d) ppm C-3 showed the presence of two double bonds. The HMBC spectral analysis (Fig. 2) displayed correlation peaks between H-13 and C-3, C-4 and C-5; H-3 and C-13, C-5; H-5 and C-3, C-4 and C-13; H-6 and C-4 and C-5. These data clearly indicated the presence of $CHCCH_3CHCH$. ^1H and ^{13}C NMR spectral data also indicated the existence of one oxygenated methine group (δ_H 3.83 m, and δ_C 65.5 d), two methene groups (δ_H 1.77, dd, 9.6, 15.0, 1.94, dd, 6.6, 15.0 Hz; 1.89, dd, 9.6, 14.4, 2.29, dd, 6.0, 14.4 Hz; and δ_C 41.1, t; 42.4, t). The COSY spectrum of **1** revealed that the oxygenated methine group (H-10) connected with these two methene groups (H-9 and H-11), while both of these two methene groups were attached to a quaternary carbon (Table 1). In addition, ^{13}C NMR spectrum showed the presence of two oxygenated quaternary carbons at δ_C 83.0 (C-7), and 90.2 ppm (C-12), one carboxylic carbon at δ_C 181.5 ppm (C-14), and one carboxyl ester carbon at δ_C 173.9 ppm (C-2). With two of the six degrees of unsaturation required by the molecular formula assigned to two double bonds, and two carboxyl groups, the two remaining degrees of unsaturation were ascribed to two cyclic systems. One was carbocyclic, the other was the oxygencyclic. The HMBC correlations shown by the methyl groups at δ_H 1.11, H-15; 1.38, H-16 (Table 1 and Fig. 2) indicated that 15-Me and the carboxylic group connected at the same quaternary carbons at δ_C 53.6 ppm (C-8), and this carbon was connected with one of the methylene groups (C-9, δ_C 41.1 ppm) and the oxygenated quaternary carbon at δ_C 83.0 ppm (C-7); while the 16-Me group attached to the other methylene groups (C-11, δ_C 42.4 ppm) and the oxygenated quaternary carbon at δ_C 90.2 ppm (C-12). Furthermore, in the HMBC spectrum, both H-5 (δ_H 7.84) and H-6 (δ_H 6.26) showed cross peaks with the oxygenated quaternary carbon at δ_C 83.0 ppm (C-7) revealed that the fragment $CHCCH_3CHCH$ connected with δ_C 83.0 ppm (C-7) through C-6 (δ 128.7). Since the carboxyl ester carbon (δ 173.9) only showed cross peak with H-3 (δ 5.91) in the HMBC spectrum, it was shown that δ 173.9 (C-2) connected with the fragment $CHCCH_3CHCH$ by C-3 (δ 127.2). Thus, the skeleton of compound **1** was confirmed, which was a very unique sesquiterpene lactone.

The coupling constants of these two methene groups (δ_H 1.77, dd, 9.6, 15.0, H-9e; 1.94, dd, 6.6, 15.0 Hz, H-9a; 1.89, dd, 9.6, 14.4, H-11e; 2.29, dd, 6.0, 14.4 Hz, H-11a) suggested the presence of a 10β-hydroxyl group. An NOE difference experiment was performed to determine the stereochemistry of compound **1** and clarify the structural assignment. When H-10 was saturated, two significant positive signals at δ_H 1.89 (H-11e) and 1.77 (H-9e) were observed, confirming the β configuration of the 10-hydroxyl group. When H-15 was saturated, four significant positive signals at δ_H 7.84 (H-5), 6.26 (H-6), 1.94 (H-9a), and 1.77 (H-9e) were observed. According to the three-dimensional structure model, this indicated that the 15-methyl group was in the β configuration, while the 7-hydroxyl group was in the α configuration. In addition, when H-16 was saturated, four significant positive signals at δ_H 7.84 (H-5), 6.26 (H-6), 2.29 (H-11a), and 1.89 (H-11e) were observed. This confirmed the α configuration of the 7-hydroxyl group, and also indicated that the 16-methyl group was in the β configuration. Thus, the structure of compound **1** was

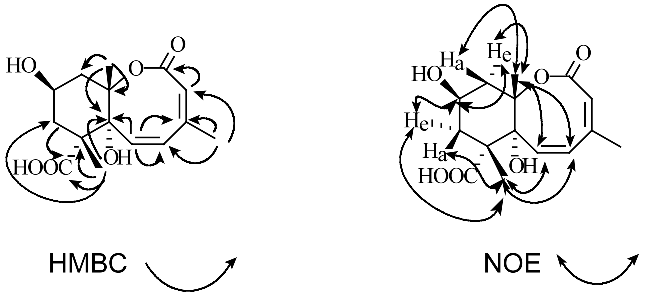

Figure 2. Significant HMBC (H→C) and NOE correlations of compound **1**.

deduced as shown (Fig. 1) and named amygdalactone. The complete interpretation of the NMR data was based on the results of COSY, HMQC, HMBC, and NOE experiments (Table 1). Compound **1** is the first case of sesquiterpene that has a cyclohexa[7,12-*g*]octalactone ring system.

Sesquiterpene lactones are natural products occurring in many plant families. These compounds are known for their various biological activities, including cytotoxicity to tumor cells,[5] insect antifeedant and repellent effects.[6,7] The cytotoxicity of compound **1** toward K562, P3HR-1 and CEM leukemia cancer cell lines was evaluated and the results showed very low inhibition rate at 9.63%, 9.47% and 7.83% at a concentration of 10 μg/mL, respectively.

Acknowledgements

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4. Compound **1**: (6 mg, 0.0012%) amorphous solid, $[\alpha]_D^{24}$ +4.3 (*c* 0.06, $CHCl_3$); UV (MeOH) λ_{max} nm (log ε) 203 (3.48), 255 (3.78). IR (film) ν_{max} 3395, 1760, 1692, 1601, 1600, 1464, 1377, 1260, 1047, 941 cm^{-1}.
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