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An unusual diterpene glycoside from the nuts of almond (*Prunus amygdalus* Batsch)

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Abstract—A new unusual kauranoid diterpene glycoside, named amygdaloside, was isolated from the nuts of almond (*Prunus amygdalus*). It's structure was established as 17-*O*-β-D-glucopyranoside *ent*-6,7-epoxy-6-hydroxyl-6,7-secokaur-19-oic acid, 6,19-lactone 16β17-diol on the basis of high-resolution 1D and 2D NMR spectral studies.

Nutraceuticals are naturally-derived, bioactive (usually phytochemical) compounds that have health promoting, disease preventing or medicinal properties. Nutraceutical compounds or substances can be delivered in the form of food (functional food) or as a dietary supplement, or in both forms.[1] In recent years, both the agri-food industry and individual consumers have looked to food not only for basic nutrition, but also for health benefits. These considerations prompted us to undertake systematic studies on the bioactive constituents of almonds (*Prunus amygdalus* Batsch), which belong to the Rosaceae family that also includes apples, pears, prunes, and raspberries.[2] Almonds are one of the most popular tree nuts on a worldwide basis and rank number one in tree nut production. They are typically used as snack foods and as ingredients in a variety of processed foods, especially bakery and confectionery products. The United States is the largest almond producer in the world. In this report, we describe the isolation and structural elucidation of amygdaloside, which has an unusual B ring-cleaved kauranoid skeleton (**1**) from the nuts of almond.

The ethanol extract of almond nuts was chromatographed successively on silica gel and RP C-18 columns to afford compound **1** (35 mg).[3] It's structure was established by assignments of 1D and 2D NMR spectra supported by MS data.

Compound **1** has the molecular formula $C_{26}H_{40}O_{10}$, established by positive-ion HRFAB-MS (m/z 513.2688, $[M+H]^+$; calcd for $C_{26}H_{41}O_{10}$: 513.2699) as well as ^{13}C and DEPT NMR spectral data (Table 1). It's IR spectrum displayed characteristic absorptions for hydroxyl groups (3400–3500 cm^{-1}), a glycosidic linkage (1000–1100 cm^{-1}), and a five-membered lactone (1759 cm^{-1}). A total of 26 carbon signals were observed in the ^{13}C NMR spectrum, six of which could be assigned to the terminal glucopyranoside. The identification of **1** as a diterpenoid derivative was supported by the remaining carbon resonances and associated DEPT signals: two methyl groups (δ_C 17.9 and 27.8), nine methylene groups (δ_C 15.7, 19.1, 23.7, 26.1, 31.3, 37.4, 50.4, 75.1 and 75.2), two of which carried oxygen atoms (δ_C 75.1 and 75.2), four methine groups (δ_C 45.8, 50.5, 57.6 and 103.6), one of which was a hemiacetal functionality (δ_C 103.6), four quaternary carbons (δ_C 38.7, 44.3, 50.5 and 81.0) including one oxygenated carbon (δ_C 81.0), and one ester carbonyl carbon (δ_C 179.4). This hypothesis was confirmed by the ^1H NMR spectrum of **1**, which showed two tertiary methyl groups at δ_H 1.03 (s) and

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1.38 (s), four oxygenated methylene protons at δ_H 3.62 (1H, d, $J=12.8$ Hz), 3.96 (1H, d, $J=11.4$ Hz), 4.47 (2H, d, $J=11.6$ Hz), and one hemiacetal proton at δ_H 5.90 (1H, d, $J=8.8$ Hz).

In addition to the chemical shift trends, the 2D COSY, TOCSY, HMQC and HMBC NMR spectra (Fig. 2 and Table 1), verified that **1** possessed most of the structural elements of a kauranoid lactone framework. However, the chemical shifts attributed to H-6 (δ_H 5.90 1H, d, $J=8.8$ Hz) and C-6 (δ_C 103.6) as well as H-7 (δ_H 4.47 1H, d, $J=11.6$ Hz and 3.62 1H, d, $J=12.8$ Hz) and C-7 (δ_C 75.1) were significantly downfield from their expected values. This trend, the absence of a COSY correlation between the H-6 and H-7 signals, and the presence of (H-6, C-7) and (H-7, C-6) cross peaks in the HMBC spectrum suggested that the C_6–C_7 bond was cleaved and replaced by one intervening oxygen atom. Thus, the structure of the aglycon part of compound **1** was confirmed to be an unusual B ring-cleaved kauranoid skeleton.

Table 1. NMR Spectral data for compound **1** (C_5D_5N) (δ in ppm, J in Hz)

	^{1}H	^{13}C	TOCSY	ROESY
1	1.52 m 0.95 m	31.3 t	H-2, 3	2, 3, 9
2	1.65 m 1.57 m	15.7 t	H-1, 3	
3	1.67 m 1.56 m	23.7 t	H-1, 3	
4		44.3 s		
5	2.47 d 8.8	50.5 d	H-6	7, 9, 18
6	5.90 d 8.8	103.6 d	H-5	7, 20
7	4.47 d 11.6 3.62 d 12.8	75.1 t		5, 6, 14, 15
8		50.5 s		
9	1.13 m	57.6 d	H-11, 12, 13	1, 5, 11, 15
10		38.7 s		
11	1.54 m 1.51 m	19.1 t	H-9, 12, 13, 14	9
12	1.81 m 1.44 m	26.1 t	H-11, 13	
13	2.40 m	45.8 d	H-9, 11, 12, 14	14
14	2.24 m 2.02 d 11.6	37.4 t	H-11, 12, 13	13, 20
15	1.62 m 1.61 m	50.4 t		7, 9
16		81.0 s		
17	4.47 d 11.6 3.96 d 11.4	75.2 t		1′
18	1.38 s	27.8 q		3, 5
19		179.4 s		
20	1.03 s	17.9 q		2, 6, 14
1′	5.03 d 8.0	106.5 d	H-2, 3, 4, 5, 6	17, 3′
2′	4.09 t 7.2	75.5 d	H-1, 3, 4, 5, 6	
3′	4.24 m	78.8 d	H-1, 2, 4, 5, 6	
4′	4.24 m	71.7 d	H-1, 2, 3, 5, 6	
5′	4.00 m	78.6 d	H-1, 2, 3, 4, 6	
6′	4.42 m 4.59 br 11.2	62.8 t	H-1, 2, 3, 4, 5	

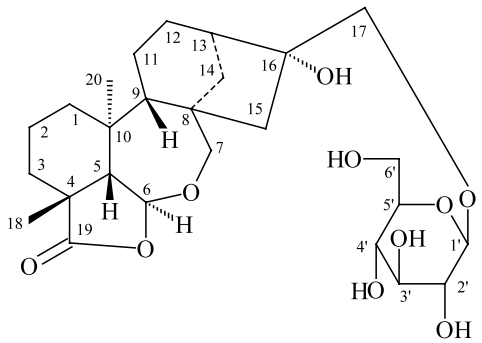

Figure 1. Molecular structure of amygdaloside.

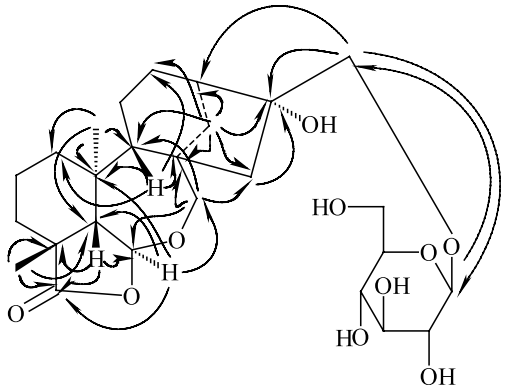

Figure 2. Significant HMBC (H→C) correlations of compound **1**

The ^{1}H NMR spectrum of **1** also displayed one anomeric proton at δ 5.02, d, $J=7.2$ Hz. The ^{13}C NMR spectrum exhibited signals corresponding to a glucopyranose unit (δ 106.5, d, C-1′; δ 75.5, d, C-2′; δ 78.8, d, C-3′; δ 71.7, d, C-4′; δ 78.6, d, C-5′; and δ 62.8, t, C-6′). The β-anomeric configuration for the glucose was deduced from its large $^3J_{H1,H2}$ coupling constants ($J=$ 8.0 Hz).[4] An (H-1′, C-17) HMBC correlation suggested that the β-glucopyranose unit was attached at the C-17 position of compound **1**. This hypothesis was further confirmed by a cross peak between H-1′ and H-17 in the ROESY spectrum.

The relative stereochemistry of **1** was assigned on the basis of the ROESY spectrum (Table 1). The ROESY correlations observed for H-20 with both H-6 and H-14 and for H-5 with both H-9 and H-18 showed *cis* relationships among H-5, H-9, and H-18 and also among H-6, H-14, and H-20. Thus, the stereochemistry at positions 4, 5, 8, 9, 10 and 13 corresponded to an *ent*-kauranoid. The relative configuration of the hydroxyl group at position 16 was determined to be β by comparing the ^{13}C data for **1** with related kauranoid diterpenes having the same structures of ring C and ring D.[5–7] Thus, compound **1** was determined as 17-*O*-β-D-glucopyranoside *ent*-6,7-epoxy-6-hydroxyl-6,7-secokaur-19-oic acid, 6,19-lactone 16β17-diol (Fig. 1).

There are three types of ring B-cleaved kaurene skeleton. The first type is exemplified by the enmein type

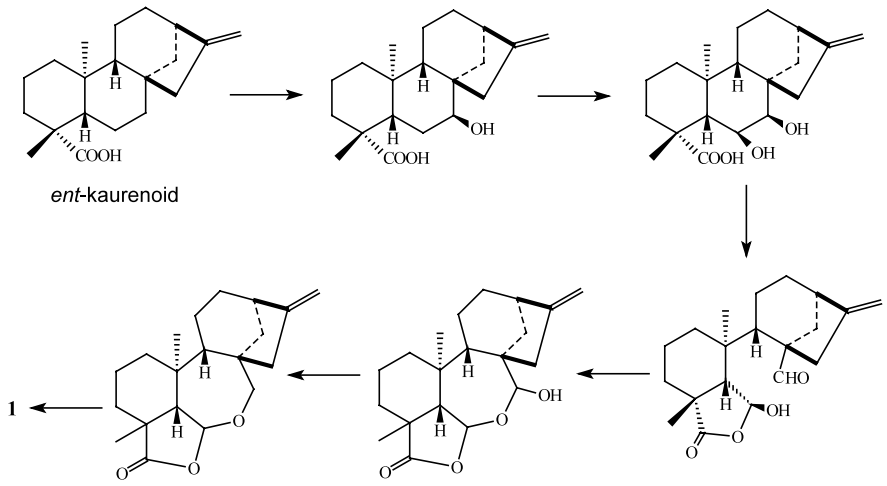

Figure 3. Proposed biosynthetic pathway from *ent*-kaurenoid to compound **1**.

diterpenoids, which have the 6,20-epoxy and 1,7-lactone structures and found in the bitter constituents of the plants of genus *Isodon* and *Robdosa*.[8–10] The second type includes the fujenal type diterpenoids, previously reported as secondary metabolites of the fungus *Gibberella fujikuroi*.[11,12] These latter types of compounds have 6,19-lactone or anhydride and 7-aldehyde structures. However, they have not been reported in higher plants. Amygdaloside represents yet the third type of ring B-cleaved kaurene skeleton, distinguished by 6,7-epoxy 6,7-*seco* structures. So far only three this type compounds have been reported from the French liverwort *Jungermannia exsertifolia* ssp. *cordifolia*.[13] However, amygdaloside is the first example of a naturally occurring diterpene glycoside with such a carbon skeleton, and *Prunus amygdalus* Batsch is the second source of this type ring B-cleaved kaurene skeleton.

The tetracyclic diterpenoid *ent*-kaurane is regarded as an intermediate in the biogenesis of enmein and fujenal.[14,15] In an analogous fashion, amygdaloside may be presumed to originate from the modification of ring B of ent-kaur-16-en-19-oic acid (Fig. 3).

The kaurane diterpenoids, a large and important group of terpenes, have been found to be widespread in families such as Labiatae, Compositae and Euphorbiaceae. Many kauranoid derivatives have been reported as active principles of natural origin for anti-HIV,[16] antiinflammatory,[17] antitumor,[17] antibacterial,[17,18] and analgesic[19] drugs. An evaluation of the cytotoxicity of compound **1** toward U937 and CEM leukemia cancer cell lines, which are known to be sensitive to many cytotoxic drugs, showed very low inhibition rates of 6.54 and 16.16% at a concentration of 10 μg/mL, respectively.

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3. The dried almond nut powders (14 kg) (Almond nuts were supplied by the California Almond Board) were extracted successively with hexane (10 L×2) and 95% EtOH (10 L×3) at room temperature. After evaporation of ethanol in vacuo, the residue (60 g) was first subjected to silica gel column chromatography with a hexane–ethyl acetate (6:1) solvent system to give fractions 1–3, then with ethyl acetate to give fraction 4, and finally with ethyl acetate–MeOH–H_2O (20:1.2:0.8, 10:1.2:0.8, 5:1.2:0.8 and 0:0:1) solvent mixtures to give fractions 5–11. Fraction 7 eluted by ethyl acetate–MeOH–H_2O (10:1.2:0.8) was subjected to reverse-phase C-18 column chromatography with 65% MeOH to give compound **1** (35 mg, yield 0.00025%). **Amygdaloside** (**1**): white amorphous solid; Mp 193–196°C; $[\alpha]_D^{25}$ −44.9° (MeOH, *c* 0.01); UV (MeOH) [λ_{max} (ε)] 245 (175), 251 (196), 256 (205), 262 (158); IR_{max}^{KBr} cm^{-1}: 3400–3500, 2934, 2868, 1759, 1457, 1157, 1075, 944. APCI-MS, *m*/*z* 535 $[M+Na]^+$; HRFAB-MS *m*/*z* 513.2688 $[M+H]^+$ (calcd for $C_{26}H_{41}O_{10}$ 513.2699); ^1H NMR (C_5D_5N, 600 MHz) and ^{13}C NMR (C_5D_5N, 150 MHz) data (Table 1).
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