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New Prenylated Benzoic Acid and Other Constituents from Almond Hulls (*Prunus amygdalus* Batsch)

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One new prenylated benzoic acid derivative, 3-prenyl-4-*O*-β-D-glucopyranosyloxy-4-hydroxylbenzoic acid, and three known constituents, catechin, protocatechuic acid, and ursolic acid, have been isolated from the hulls of almond (*Prunus amygdalus*). Complete assignments of the proton and carbon chemical shifts for the new prenylated benzoic acid derivative were accomplished on the basis of high-resolution 1D and 2D nuclear magnetic resonance data. All of these compounds except ursolic acid are being reported from almond hulls (*P. amygdalus*) for the first time.

KEYWORDS: Almond hulls; *Prunus amygdalus*; prenylated benzoic acid derivative

INTRODUCTION

Almonds (*Prunus amygdalus* Batsch) belong to the Rosaceae family that also includes apples, pears, prunes, and raspberries (*1*). Almond is one of the most popular tree nuts on a worldwide basis and ranks number one in tree nut production. They 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 U.S. almonds are grown in California in an area that stretches over 400 miles from Bakersfield to Red Bluff (*2*). Over 7000 individual growers cultivate more than 400 000 acres. Almonds are California's largest tree crops based on dollar value, acreage, and world distribution. Five major varieties of almonds grown in California include Nonpareil, Mission, California, Ne Plus Ultra, and Peerless. Of the five groups listed, most almond production (about 90%) falls into three major marketing categories of Nonpareil, California, and Mission. 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 (*Prunus dulcis* (Mill.) D. A. Webb) are a rich source of three 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 from almond hulls prompted us to investigate the chemical composition of the hulls systematically. In this report, we described the isolation and structure elucidation of a new prenylated benzoic acid derivative (**1**), together with three known constituents, which include catechin (**2**), protocatechuic acid (**3**), and ursolic acid (**4**), isolated from the hulls of almond (*P. amygdalus*).

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MATERIALS AND METHODS

General Procedures. Optical rotations were obtained on a JASCO P-1020 polarimeter. Fourier transform infrared spectroscopy (FT-IR) was performed on a Magna 550 spectrometer. ^{1}H (600 MHz), ^{13}C (150 MHz), and all 2D nuclear magnetic resonance (NMR) spectra were run on a Varian AM-600 NMR spectrometer, with tetramethylsilane as the internal standard. Atmospheric pressure chemical ionization mass spectrometry (APCIMS) was obtained on a Fisons/VG Platform II mass spectrometer. Thin-layer chromatography (TLC) was performed on Sigma-Aldrich TLC plates (250 μm thickness, 2−25 μm particle size), with compounds visualized by spraying with 5% (v/v) H_2SO_4 in ethanol solution.

Plant Material. Almond hulls were supplied by the California Almond Board. A voucher specimen (HS17) was deposited in the Department of Food Science, Cook College, Rutgers University.

Extraction and Isolation Procedures. The dried almond hulls (450 g) were extracted with 95% ethanol (4 L) at 50 °C for 1 day. The extract was concentrated to dryness under reduced pressure, and the residue was suspended in water (250 mL) and partitioned successively with ethyl acetate (3 × 250 mL) and *n*-butanol (3 × 250 mL). The ethyl acetate fraction was subjected to silica gel column chromatography with a $CHCl_3$−MeOH (40:1) solvent system first to give fractions 1−3 and then with a $CHCl_3$−MeOH−H_2O (5:1:0.15−2:1:0.2) solvent system to give fractions 4−8. Fraction 2 eluted by $CHCl_3$−MeOH (40:1) was subjected to Sephadex LH-20 column chromatography with 95% EtOH to give compound **4** (120 mg). Fraction 5 eluted by $CHCl_3$−MeOH−H_2O (5:1:0.15) was subjected to Sephadex LH-20 column chromatography with 95% EtOH to give 5 fractions (5−1 to 5−5). Fraction 5−5 was subjected to a prepared TLC plate eluted by $CHCl_3$−MeOH−H_2O (5:1:0.15) and then rechromatographed on Sephadex LH-20 eluted by 95% EtOH to afford 430 mg of compound **2**. Fraction 6 eluted by $CHCl_3$−MeOH−H_2O (4:1:0.2) was subjected to Sephadex LH-20 column chromatography with 95% EtOH to give 4 fractions (6-1 to 6-4). Fraction 6-3 was isolated by a prepared TLC plate eluted by ethyl acetate−MeOH−H_2O (10:1:1) to give compound **3** (30 mg). Fraction 6-4 was rechromatographed on Sephadex LH-20 eluted by 95% EtOH to afford 15 mg of compound **1**.

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Spectral Identification of Known Compounds. *Catechin (2).* White powder; $[\alpha]_D^{25}$ 15.1° (acetone, *c* 0.5). APCIMS *m/z*: 289 $[M - H]^-$. ^1H NMR (CD_3OD, 600 MHz): δ 2.53 (dd, J = 16.1, 8.2 Hz, H-4), 2.87 (1H, dd, J = 16.1, 5.2 Hz, H-4), 4.02 (1H, m, H-3), 4.60 (1H, d, J = 7.3 Hz, H-2), 5.87 (1H, d, J = 2.2 Hz, H-6), 5.96 (1H, d, J = 2.2 Hz, H-8), 6.74 (1H, dd, J = 1.5, 8.4, H-6′), 6.78 (1H, d, J = 8.4, H-5′), 6.85 (1H, d, J = 1.5 Hz, H-2′). ^{13}C NMR (150 MHz, CD_3OD): δ 28.4 (t, C-4), 68.8 (d, C-3), 82.8 (d, C-2), 95.7 (s, C-8), 96.5 (d, C-6), 101.0 (s, C-4a), 115.3 (d, C-2′), 116.3 (d, C-5′), 120.2 (d, C-6′), 132.2 (s, C-1′), 146.2 (s, C-3′, 4′), 156.9, 157.5, 157.8 (s, C-5, C-7, C-8a) [identical with the literature (*4*)].

Protocatechuic Acid (3). White powder. APCIMS *m/z*: 153 $[M - H]^-$. ^1H NMR (CD_3OD, 600 MHz): δ 6.75 (1H, d, J = 8.0 Hz, H-5), 7.39 (1H, dd, J = 2.0, 8.0 Hz, H-6), 7.43 (1H, d, J = 2.0 Hz, H-2) [identical with the literature (*5*)].

Ursolic Acid (4). White powder. APCIMS *m/z*: 455 $[M - H]^-$. ^1H NMR (C_5D_5N, 600 MHz): δ 5.50 (1H, brs, H-12), 3.43 (1H, m, H-3), 2.52 (1H, d, J = 11.0 Hz, H-18), 1.24 (3H, s, H-23), 1.22 (3H, s, H-27), 1.05 (3H, s, H-26), 1.02 (3H, s, H-24), 0.99 (3H, d, J = 6.1 Hz, H-30), 0.94 (3H, d, J = 6.2 Hz, H-29), 0.88 (3H, s, H-25). ^{13}C NMR (C_5D_5N): δ 15.7 (q, C-24), 16.6 (q, C-25), 17.4 (q, C-26), 17.5 (q, C-29), 18.8 (t, C-6), 21.4 (q, C-30), 23.6 (t, C-11), 23.8 (q, C-27), 24,9 (t, C-16), 28.1 (t, C-2), 28.7 (t, C-15), 28.8 (q, C-23), 31.1 (t, C-21), 33.6 (t, C-7), 37.3 (t, C-22), 37.4 (s, C-10), 39.1 (d, C-20), 38.4 (t, C-1), 38.4 (s, C-4), 39.5 (d, C-19), 40.0 (s, C-8), 42.5 (s, C-14), 48.0 (s, C-17), 53.5 (d, C-18), 55.8 (d, C-5), 78.1 (d, C-3), 125.6 (d, C-12), 139.3 (s, C-13), 180.0 (s, C-28) [identical with the literature (*6*)].

RESULTS AND DISCUSSION

The ethyl acetate fraction of almond hulls extract was chromatographed successively on Silica gel, Sephadex LH-20, and a prepared TLC plate to afford one new compound and three known compounds. Their structures were established by interpretation and full assignments of 1D and 2D NMR spectroscopic data and comparison with literature data.

Compound **1**, an amorphous solid, was assigned a molecular formula of $C_{18}H_{24}O_8$ determined by negative-ion APCIMS ($[M - H]^-$ at *m/z* 367) as well as from its ^{13}C NMR data. Its IR spectrum indicated the presence of hydroxyl groups (3410 cm^{-1}), carboxyl groups (1680 cm^{-1}), and aromatic groups (1600, 1510, and 990 cm^{-1}). The splitting pattern in the ^1H NMR spectrum of the three aromatic protons [δ_H 7.83 (dd, J = 2.4, 9.0 Hz), 7.79 (d, J = 2.4 Hz), and 7.17 (d, J = 9.0 Hz)] established that the aromatic ring was a 1,3,4-trisubstituted benzene ring. In the HMBC spectrum of compound **1** (**Table 1**), the proton signal at δ_H 7.79 (H-2) showed cross-peaks to a carbonyl carbon at δ_C 170.2 and a hydroxy-bearing quaternary carbon at δ_C 160.4 (C-4) and with a protonated carbon at δ_C 130.4 (C-6) and a methylene carbon at δ_C 29.1 (C-1′), which was considered to be located on the side chain, suggesting that compound **1** is a 3-substituted 4-hydroxybenzoic acid derivative. This was supported by the ^{13}C NMR spectral data at δ_C 132.1, C-1; 132.0, C-2; 125.5, C-3; 160.4, C-4; 115.0, C-5; 130.4, C-6; and 170.2, COOH. In addition, both the ^1H and the ^{13}C NMR spectra showed the presence of a prenyl group. In the ^1H NMR spectrum, two singlet methyl groups at δ 1.72 and 1.75 ppm, one olefinic proton at δ 5.34 ppm, and one methylene group at δ 3.31 ppm were observed. This was supported by the appropriate resonances in the ^{13}C NMR spectrum (δ 29.1, C-1′; 123.1, C-2′; 134.0, C-3′; 17.9, C-4′; and 25.9, C-5′). Thus, the side chain of the 3-substituted 4-hydroxybenzoic acid derivative is the prenyl group. The location of the prenyl group was also proved by the cross-peak between H-1′ (δ 3.31) and C-2 (δ 132.0) and C-4 (δ 160.4) in the HMBC spectrum of compound **1**.

Table 1. NMR Spectral Data for Compound **1** (CD_3OD) (δ in ppm, J in Hz)

	^1H	^{13}C		^1H	^{13}C
COOH		170.2 s	4′	1.72 s	17.9 q
1		132.1 s	5′	1.75 s	25.9 q
2	7.79, d, 2.0	132.0 d	glucose		
3		125.5 s	1″	5.02, d, 7.2	101.8 d
4		160.4 s	2″	3.53 m	74.9 d
5	7.17, d, 9.0	115.0 d	3″	3.48 m	78.2 d
6	7.83, dd, 2.0, 9.0	130.4 d	4″	3.41 m	71.3 d
1′	3.31 m	29.1 t	5″	3.39 m	78.2 d
2′	5.34 m	123.1 d	6″	3.71, dd, 5.4, 12.0	62.5 t
3′		134.0 s		3.90, dd, 2.4, 12.0	

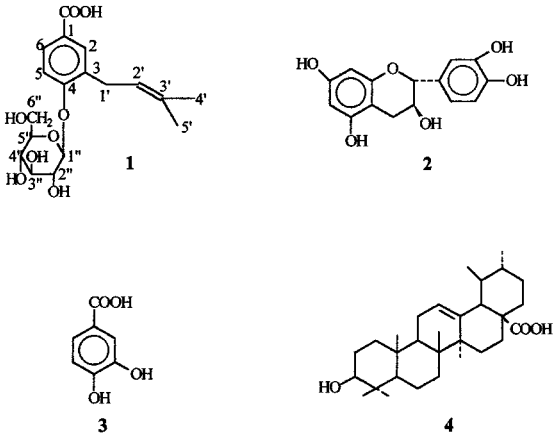

Figure 1. Structures of compounds **1**–**4**.

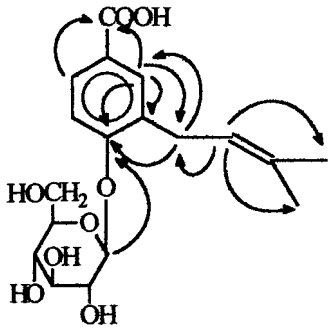

Figure 2. Significant HMBC (H→C) correlations of compound **1**.

Furthermore, the ^1H NMR spectrum of **1** showed one anomeric proton at δ 5.02, d, J = 7.2 Hz. The ^{13}C NMR spectrum exhibited the signals for the glucopyranose unit (δ 101.8, d, C-1″; δ 74.9, d, C-2″; δ 78.2, d, C-3″; δ 71.3, d, C-4″; δ 78.2, d, C-5″; and δ 62.5, t, C-6″). The β-anomeric configuration for the glucose was judged from its large $^3J_{1,2}$ coupling constants (J = 7.2 Hz) (*7*). HMBC correlation between H-1″/C-4 suggested that the β-glucopyranose unit was attached at the C-4 position of the 3-substituted 4-hydroxybenzoic acid derivative. Thus, compound **1** was determined as 3-prenyl-4-*O*-β-D-glucopyranosyloxy-4-hydroxylbenzoic acid (**Figure 1**). Full assignments of the ^1H and ^{13}C NMR signals were accomplished using HMBC (**Figure 2**), HMQC, $^1H-^1H$ COSY, and TOCSY experiments (**Table 1**).

In addition to the new prenylated benzoic acid derivative, three known constituents, **2**−**4**, have also been isolated in this study. Their structures were identified by comparison of their NMR and MS data with those reported in the literature (*4*−*6*). All of these compounds except ursolic acid are being reported from this species (*P. amygdalus*) for the first time. It was reported that the simple phenolic protocatechuic acid (PA) is one of the major benzoic acid derivatives from vegetables and

fruits with a strong antioxidative effect, 10-fold higher than that of α-tocopherol (*8*). PA, even at 100 ppm in a diet, shows potent chemopreventive effects on colon and oral carcinogenesis in rats (*9*). A recent study (*10*) was initially performed to estimate the effectiveness of PA against TPA-induced tumor promotion in mouse skin. Interestingly, the modulation of tumor development was apparently dependent on the dose (1.6−20 000 nmol) and timing (5 min−3 h before TPA treatment) of PA application. Catechin is the flavonoid that is the most widely distributed in edible plants and in foodstuffs derived from plants. It is mainly supplied by beverages (red wine and tea) and by some fruits such as apples. Many in vitro and animal studies have demonstrated the high antioxidant activity of catechin and its inhibitory effect on numerous enzymes, which may result in a protective activity toward cancer, cardiovascular, and inflammatory diseases (*11*−*18*). Ursolic acid, the common triterpene, has been reported to possess anti-HIV (*19*) and anticancer (*20*) activities.

It was reported that prenylated compounds had the antioxidant (*21*), anti-HIV (*22*), cytotoxic (*23*), and immunosuppressive (*24*) activities. Because compound **1** is the first prenylated compound isolated from the *Prunus* genus, further study on its bioactivities will be necessary.

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