Ecdysteroids of Quinoa Seeds (*Chenopodium quinoa* Willd.)

Nanqun Zhu,† Hiroe Kikuzaki,‡ Bret C. Vastano,† Nobuji Nakatani,‡ Mukund V. Karwe,† Robert T. Rosen,† and Chi-Tang Ho*,†

Department of Food Science and Center for Advanced Food Technology, Rutgers University, 65 Dudley Road, New Brunswick, New Jersey 08901-8520, and Department of Food and Nutrition, Osaka City University, 3-3-138 Sugimoto, Sumiyoshi, Osaka, Japan

Quinoa (*Chenopodium quinoa*) is a hardy and nutritious Latin American pseudo-cereal. Studies on the seeds led to the isolation of five ecdysteroids using column chromatography. Their structures were determined as ecdysterone, makisterone A, 24-*epi*-makisterone A, 24(28)-dehydromakisterone A, and 20,26-dihydroxyecdysone by spectroscopic methods. This study demonstrates that quinoa seeds are a source of ecdysteroids, which were reported to be molting hormones in insects.

Keywords: *Quinoa seeds; Chenopodium quinoa; ecdysteroids; NMR; molting hormones*

INTRODUCTION

Quinoa (*Chenopodium quinoa*) is a crop that has been cultivated in Latin America for centuries. It is resistant to drought and frost and can be grown in very poor soil and at high altitude. More importantly, its seeds are high in protein content and average ∼14.6% on wet basis. This protein is of an exceptionally high quality and is particularly rich in essential amino acids, such as histidine and lysine, which are deficient in most grain crops and necessary for proper amino acid nutrition in humans. Thus, the protein quality of quinoa seeds is comparable to that of whole dry milk. In terms of vitamin content, quinoa has much more riboflavin, α-tocopherol, and carotenes than barley, rice, or wheat. Moreover, quinoa has more Ca, Fe, K, Mg, Cu, and Mn than other cereals (*1*, *2*). Thus, quinoa is of considerable interest in the world. In developing countries, many people cannot afford to achieve a balanced diet, especially with regard to sufficient and good-quality protein, whereas in developed countries, quinoa could be used to improve the nutritional quality of snacks, breads, pastas, and other prepared food (*1*, *2*). Now, quinoa seeds are used in making soups or are ground into flour to prepare breads, cakes, beers, and animal feeds.

There is a big problem related to the consumption of quinoa: the seeds are covered with bitter substances that must be removed prior to consumption. Generally, these bitter substances were believed to be saponins (*3*). In previous studies, several saponins have been purified and identified (*4*, *5*), and three groups of saponins were found in quinoa, in which the three triterpenoids, oleanolic acid, hederagenin, and phytolaccagenic acid, serve as the aglycon in each group (*6*). However, the chemical components of quinoa seeds are not completely known. Here, we report for the first time the isolation of five phytoecdysteroids from quinoa seeds. Their structures were determined as makisterone A, 24-*epi*-makisterone A, 24(28)-dehydromakisterone A, 20-hydroxyecdysone, and 20,26-dihydroxyecdysone using NMR and MS spectra and verified by 2D NMR data.

* Author to whom correspondence should be addressed [telephone (732) 932-9611, ext 235; fax (732) 932-6776; e-mail ho@aesop.rutgers.edu].

† Rutgers University.

‡ Osaka City University.

MATERIALS AND METHODS

Materials. Quinoa seeds were purchased from Quinoa Corp. (Torrance, CA). Negative APCI-MS spectra were obtained on a Micromass Platform II system (Micromass Co., Beverly, MA) equipped with a Digital DECPC XL560 computer for data analysis. ^{1}H NMR, ^{13}C NMR, ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY spectra were recorded on a Varian U-500 spectrometer. Chemical shifts are expressed in parts per million (δ) using tetramethylsilane (TMS) as internal standard. All solvents and TLC plates (250 μm thickness, 2−25 μm particle size) were purchased from Fisher Scientific (Springfield, NJ). Spots were observed under a UV detector (254 nm) and visualized by 10% H_2SO_4 ethanol solution followed by heating. Silica gel (130−270 mesh), RP-18 gel, Sephadex LH-20, and Diaion HP-20 gel were purchased from Aldrich Chemical Co. (Milwaukee, WI).

Extraction and Isolation. Dried seeds (4 kg) were extracted twice for 3 days each, with 95% aqueous ethanol at room temperature. The extracts then were evaporated to dryness (120 g), and the residue was suspended in water and partitioned successively with hexane (20 g), ethyl acetate (10 g), and butanol (35 g). The butanol fraction was subjected to column chromatography on Diaion HP-20 gel to give four [water, waterr/ethanol (3:7), water/ethanol (1:9), and acetone] fractions. On the basis of the TLC analysis data of the resulting elution solvent, the water/ethanol (3:7) fraction was separated into four fractions by Sephadex LH-20 column chromatography with 90% aqueous ethanol. The second of these fractions was repeatedly separated by column chromatography (silica gel) and eluted with ethyl acetate/methanol/water (20:1:0.8) to give several subfractions (1−6). Subfraction 2 was purified over silica gel chromatography using ethyl acetate/methanol/water/hexane (25:1:0.8:2.5), affording compounds **1** (35 mg, 0.00088%) and **2** (15 mg, 0.00038%). Subfraction 1 was purified by silica gel chromatography using ethyl acetate/methanol/water/hexane (25:1:0.8:4) to give compound **3** (13 mg, 0.00033%). Subfraction 3 was purified by an RP-18 reverse phase column (60% methanol in H_2O), giving compound **4** (120 mg, 0.003%). Compound **5** (11 mg, 0.00028%) was isolated from subfraction 4 by silica gel column chromatography using ethyl acetate/methanol/water (12:1:0.8) as the eluent. In the following, asterisks indicate chemical shifts that are interchangeable in each compound.

*Makisterone A (**1**):* amorphous powder; APCI-MS, *m*/*z* 493 $[M - H]^-$; ^{1}H NMR (C_5D_5N) δ 1.05 (3H, s, H-19), 1.06 (3H, d,

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Table 1. ^{13}C NMR Spectral Data of Ecdysteroids [Compounds 1, 2 (*8*), 3 (*9*), and 5 (*11*) Were Dissolved in C_5D_5N; Compound 4 (*10*) Was Recorded in DMSO-d_6][a]

	δ				
no.	**1**	**2**	**3**	**4**	**5**
1	37.9 t	38.0 t	38.0 t	36.8 t	38.0 t
2	68.0 d	68.1 d	68.0 d	66.9 d	68.0 d
3	68.1 d	68.1 d	68.1 d	66.7 d	68.1 d
4	32.4 t	32.5 t	32.4 t	31.7 t	31.7 t
5	51.4 d	51.4 d	51.4 d	50.3 d	51.4 d
6	203.5 s	203.5 s	203.5 s	202.9 s	203.5 s
7	121.6 d	121.6 d	121.7 d	120.7 d	121.6 d
8	166.1 s	166.2 s	166.1 s	165.4 s	166.1 s
9	34.4 d	34.4 d	34.4 d	33.4 d	34.4 d
10	38.6 s	38.6 s	38.7 s	37.8 s	38.6 s
11	21.1 t	21.1 t	21.1 t	20.6 t	21.1 t
12	31.8 t	31.7 t	31.8 t	31.1 t	31.9 t
13	48.1 s	48.1 s	48.1 s	47.0 s	48.1 s
14	84.1 s	84.1 s	84.1 s	83.1 s	84.1 s
15	32.0 t	32.0 t	32.0 t	30.5 t	31.7 t
16	21.3 t	21.5 t	21.4 t	20.5 t	21.4 t
17	49.9 d	49.9 d	50.0 d	50.3 d	50.1 d
18	17.9 q	17.9 q	17.9 q	17.3 q	17.9 q
19	24.4 q	24.5 q	24.4 q	24.1 q	24.4 q
20	76.9 s	77.0 s	76.7 s	75.8 s	76.8 s
21	21.6 q	21.3 q	21.5 q	21.1 q	21.7 q
22	74.6 d	76.1 d	78.0 d	76.3 d	77.6 d
23	34.6 t	35.1 t	34.7 t	26.2 t	26.8 t
24	41.8 d	43.5 d	156.2 s	41.5 t	37.6 t
25	72.0 s	72.2 s	72.1 s	68.9 s	72.6 s
26	26.5* q	26.9* q	30.1* q	29.2* q	70.9 q
27	28.2* q	29.0* q	30.8* q	29.1* q	24.5 q
28	15.4 q	16.7 q	109.5 t		

[a] An asterisk indicates chemical shifts that are interchangeable in each compound; q = CH_3; t = CH_2; d = CH; s = C.

J = 6.5 Hz, H-28), 1.23 (3H, s, H-18), 1.29* (3H, s, H-26), 1.31* (3H, s, H-27), 1.58 (3H, s, H-21), 3.58 (1H, m, H-9), 3.97 (1H, brd, *J* = 10.5 Hz, H-22), 4.16 (1H, ddd, *J* = 11.0, 3.5, 3.5 Hz, H-2), 4.20 (1H, m, H-3), 6.26 (1H, d, *J* = 2.5 Hz, H-7); ^{13}C NMR, see Table 1.

*24-Epi-makisterone A (**2**):* amorphous powder; APCI-MS, *m*/*z* 493 $[M - H]^-$; ^1H NMR (C_5D_5N) δ 1.06 (3H, s, H-19), 1.21 (3H, s, H-18), 1.25 (3H, d, *J* = 7.0 Hz, H-28), 1.33* (3H, s, H-26), 1.39* (3H, s, H-27), 1.58 (3H, s, H-21), 3.59 (1H, m, H-9), 4.07 (1H, brd, *J* = 11.0 Hz, H-22), 4.17 (1H, m, H-2), 4.23 (1H, m, H-3), 6.26 (1H, d, *J* = 2.5 Hz, H-7); ^{13}C NMR, see Table 1.

*24(28)-Dehydromakisterone A (**3**):* amorphous powder; APCI-MS, *m*/*z* 491 $[M - H]^-$; ^1H NMR (C_5D_5N) δ 1.06 (3H, s, H-19), 1.21 (3H, s, H-18), 1.51* (3H, s, H-26), 1.57* (3H, s, H-27), 1.58 (3H, s, H-21), 3.59 (1H, m, H-9), 4.08 (1H, brd, *J* = 10.5 Hz, H-22), 4.16 (1H, brd, *J* = 11.0 Hz, H-2), 4.22 (1H, brs, H-3), 5.09 (1H, s, H-28), 5.27 (1H, d, *J* = 1.5 Hz, H-28), 6.26 (1H, brs, *J* = 2.5 Hz, H-7); ^{13}C NMR, see Table 1.

*20-Hydroxyecdysone (**4**):* amorphous powder; APCI-MS, *m*/*z* 481 $[M - H]^-$; ^1H NMR (DMSO-d_6) δ 0.75 (3H, s, H-18), 0.79 (3H, s, H-19), 1.03 (3H, s, H-21), 1.04* (3H, s, H-26), 1.06* (3H, s, H-27), 2.99 (1H, m, H-9), 3.06 (1H, brd, *J* = 10.5 Hz, H-22), 3.57 (1H, brs, H-2), 3.75 (1H, brs, H-3), 5.61 (1H, brs, H-7); ^{13}C NMR, see Table 1.

*20,26-Dihydroxyecdysone (**5**):* amorphous powder; APCI-MS, *m*/*z* 497 $[M - H]^-$; ^1H NMR (C_5D_5N) δ 1.05 (3H, s, H-19), 1.20 (3H, s, H-18), 1.47 (3H, s, H-27), 1.58 (3H, s, H-21), 3.58 (1H, m, H-9), 3.86 (2H, m, H-26), 3.91 (1H, brd, *J* = 11.0 Hz, H-22), 4.18 (1H, m, H-2), 4.20 (1H, m, H-3), 6.24 (1H, d, *J* = 2.0 Hz, H-7); ^{13}C NMR, see Table 1.

RESULTS AND DISCUSSION

The butanol fraction of the ethanol extract of quinoa seeds afforded a group of phytoecdysteroids via a series of separation techniques involving extraction, isolation, and identification. Due to their structural similarity, the ecdysteroids are difficult to separate without the use of

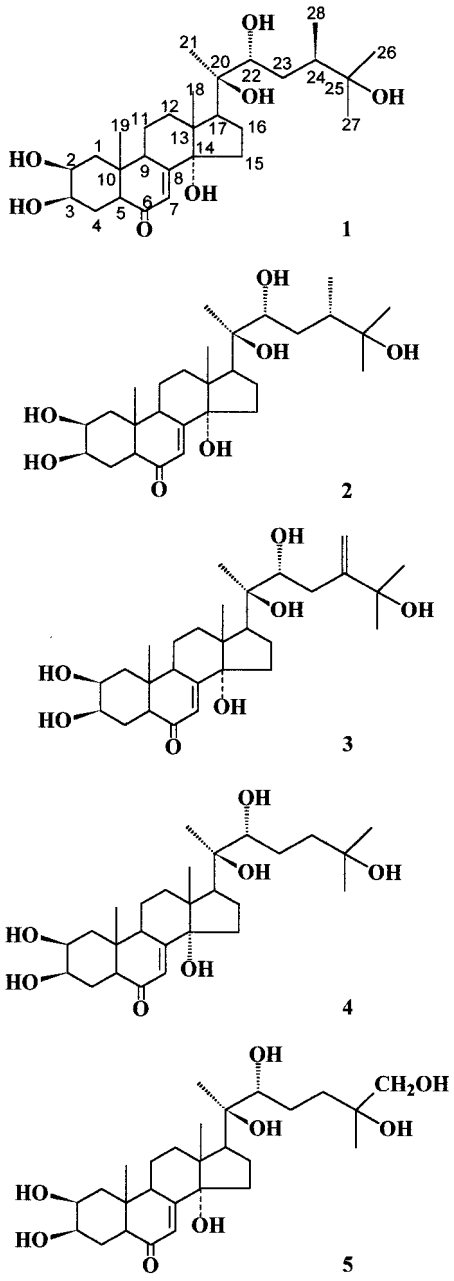

Figure 1.

HPLC (*7*). In our study, we used a new eluent system of ethyl acetate/methanol/water/hexane, which has been found to be very successful in the analysis of these ecdysteroids by simple silica gel columns. Structures of these ecdysteroids (shown in Figure 1) were determined by comparison of their MS and NMR data with reported data. They were further verified by 2D NMR techniques, including HMQC, HMBC, and NOESY spectra, which can provide much detailed information for structure determination and verification, especially for signal assignment. As shown, makisterone A (**1**), 24-*epi*-makisterone A (**2**), and 24(28)-dehydromakisterone A (**3**) belong to the group of C_{28} steroids, the only difference occurring among them being the substitution group at C-24. The stereochemistry of **1** was established as 24*R*, and **2** has 24*S* configuration (*8*), whereas compound **3** is the dehydro derivative (*9*). Compounds **4** and **5** were determined to be 20-hydroxyecdysterone (*10*) and 20,26-dihydroxyecdysone (*11*), respectively. As the most common ecdysteroid found in the plant kingdom (*8*),

20-hydroxyecdysterone is also the most abundant ecdysteroid in *C. quinoa* seeds. Generally, phytoecdysteroids have been found in many species but rarely found in seeds. Although the occurrence of ecdysteroids was indicated in the study of Dinan (*12*), this is the first report that five ecdysteroids have been isolated and identified from quinoa seeds.

On the other hand, plant steroids endowed with insect and crustacean molting hormone activity are most common in Pteridophyta (ferns), Podocarpaceae, and Amaranthaceae species (*13*). As reported, phytoecdysteroids can regulate gene activities, the metabolism of nucleic acids, protein synthesis, development, reproduction, and diapause in insects (*14*). However, the most important function of ecdysteroids is their molt inhibition activity, which has been widely examined in the strategy for insecticide research (*15*). As indicated in the study by Bergamasco and Horn (*13*), all five of these ecdysteroids are strongly active. They can disrupt normal development of several insect pests in feeding assays, and it is therefore likely that the presence of these ecdysteroids accounts for the resistance of quinoa to insect attack, which was previously believed to be the result of some toxic saponins only (*6*). In addition, phytoecdysteroids have been shown to inhibit hypercholsterolemia and hyperglyceridemia in rats (*8*), and their occurrence was possibly related to the traditional use of the genus *Palista* for sexual problems (*9*). As one of the major components of quinoa seeds, the effect of ecdysteroids on humans or animals remains of interest.

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