Oleanane Saponins in "Kancolla", a Sweet Variety of *Chenopodium quinoa*

Irene Dini, Gian Carlo Tenore, and Antonio Dini*

Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli "Federico II", Via D. Montesano, 49, 80131 Naples, Italy

Received December 7, 2001

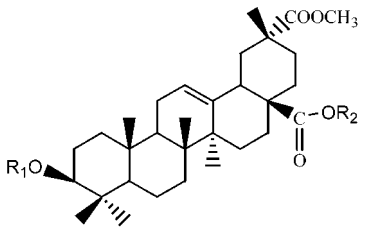
Seven triterpenoid saponins were isolated from the seeds of "kancolla", a sweet variety of *Chenopodium quinoa*. Their structures were phytolaccagenic acid 3-*O*-[α-L-arabinopyranosyl-(1″→3′)-*β*-D-glucuronopyranosyl]-28-*O*-*β*-D-glucopyranoside, oleanolic acid 3-*O*-[*β*-D-glucopyranosyl-(1″→3′)-α-L-arabinopyranosyl]-28-*O*-*β*-D-glucopyranoside, hederagenin 3-*O*-[*β*-D-glucopyranosyl-(1″→3′)-α-L-arabinopyranosyl]-28-*O*-*β*-D-glucopyranoside, phytolaccagenic acid 3-*O*-[*β*-D-glucopyranosyl-(1″→3′)-α-L-arabinopyranosyl]-28-*O*-*β*-D-glucopyranoside, oleanolic acid 3-*O*-[*β*-D-glucuronopyranosyl]-28-*O*-*β*-D-glucopyranoside, oleanolic acid 3-*O*-[α-L-arabinopyranosyl-(1″→3′)-*β*-D-glucuronopyranosyl]-28-*O*-*β*-D-glucopyranoside, and the new compound serjanic acid 3-*O*-[*β*-D-glucopyranosyl-(1″→3′)-α-L-arabinopyranosyl]-28-*O*-*β*-D-glucopyranoside (**1**). The structure of **1** was characterized on the basis of spectroscopic and chemical evidence.

"Kancolla" is the most widespread of the new varieties of quinoa (*Chenopodium quinoa* Willd.; Chenopodiaceae), a highly nutritious Andean food plant.[1−3] The seeds are the main edible part. Usually quinoa seeds contain bitter-tasting constituents (chiefly water-soluble saponins) located in the outer layers of the seed coat.[1] Because of this, they need to be washed or milled to remove the seed coat, whereas the "kancolla" seeds have a sweet taste. Nevertheless, the presence of other nonbitter saponins points to the need to process the seed, because of their unknown biological effects. These saponin constituents are considered antinutritional factors for their hemolytic capacity,[4−6] and for this reason they have to be eliminated before consumption. On the other hand, recent studies have demonstrated beneficial effects of some saponins, such as their antiallergic,[7] antidiabetic,[8,9] antiinflammatory,[10,11] antimycotic,[12−14] antiviral,[15] cancer chemopreventive,[16] hypocholesterolemic,[17,18] and immunostimulatory properties.[19] The taste and pharmacological properties are linked to the intact saponin molecules. Therefore, the aim of this work was the isolation and characterization of the intact saponins present in "kancolla" seeds. Seven triterpenoid saponins were found in "kancolla" seeds, with one of these being a new compound (**1**), and the others were isolated for the first time from this variety of quinoa. The new compound **1** is a serjanic acid derivative, an uncommon oleanane-type saponin aglycon, obtained previously only in the families Phytolaccaceae and Sapindaceae,[20−22] which could be a taxonomic marker for "kancolla". All the isolated glycosides are bisdesmosides with sugar chains made up of one or two monosaccharide units linked to C-3 and one glucose unit linked to C-28.

"Kancolla" seeds were extracted with CH_3OH. The methanolic extract was partitioned into a mixture of *n*-BuOH and H_2O to afford a BuOH-soluble portion, which was subjected to Sephadex LH-20 chromatography followed by droplet counter-current chromatography (DCCC) to give the crude saponins. The fractions were checked by TLC, and fractions 48−296 containing the crude glycosidic mixture were submitted to reversed-phase HPLC to afford the pure individual compounds. The known saponins were identified as phytolaccagenic acid 3-*O*-[α-L-arabinopyranosyl-(1″→3′)-*β*-D-glucuronopyranosyl]-28-*O*-*β*-D-glucopyranoside,[23,24] oleanolic acid 3-*O*-[*β*-D-glucopyranosyl-(1″→3′)-α-L-arabinopyranosyl]-28-*O*-*β*-D-glucopyranoside,[23] hederagenin 3-*O*-[*β*-D-glucopyranosyl-(1″→3′)-α-L-arabinopyranosyl]-28-*O*-*β*-D-glucopyranoside,[24] phytolaccagenic acid 3-*O*-[*β*-D-glucopyranosyl-(1″→3′)-α-L-arabinopyranosyl]-28-*O*-*β*-D-glucopyranoside,[23] oleanolic acid 3-*O*-[*β*-D-glucuronopyranosyl]-28-*O*-*β*-D-glucopyranoside,[25] and oleanolic acid 3-*O*-[α-L-arabinopyranosyl-(1″→3′)-*β*-D-glucuronopyranosyl]-28-*O*-*β*-D-glucopyranoside[26] by spectral data and direct comparison of their physical properties with those reported previously for these compounds.

Hederagenin 3-*O*-[*β*-D-glucopyranosyl-(1″→3′)-α-L-arabinopyranosyl]-28-*O*-*β*-D-glucopyranoside and phytolaccagenic acid 3-*O*-[*β*-D-glucopyranosyl-(1″→3′)-α-L-arabinopyranosyl]-28-*O*-*β*-D-glucopyranoside show antimicrobial activities[27] but are hemolytically inactive.[28]

Acid hydrolysis of **1** afforded deoxyphytolaccagenic acid, which was identified by comparing its ^1H and ^{13}C NMR data with reported values.[29] In compound **1**, C-28 appeared at 178.7 ppm in the ^{13}C NMR spectrum and H-18 appeared at 2.81 ppm (dd, J = 12.0 and 4.0 Hz) in the ^1H NMR spectrum, indicating the carboxyl group was glycosylated.[26] The attachment of another glycosidic chain at C-3 was suggested by the significant downfield shift observed for this carbon to the corresponding signal in other serjanic acid derivatives and was subsequently confirmed by 2D NMR experiments.



	R_1	R_2
1	β-D-Glc^3→^{1}α-L-Ara	β-D-Glc
1a	β-D-Glc^3→^{1}α-L-Ara	H

Compound **1** was obtained as a white powder (40.1 mg) that was determined to have the elemental composition $C_{48}H_{76}O_{19}$ by HRFABMS and ^{13}C NMR analysis. The IR

* To whom correspondence should be addressed. Tel: (039) 81-678535. Fax: (039) 81-678552. E-mail: andini@cds.unina.it.

10.1021/np010625q CCC: $22.00
Published on Web 05/29/2002

Table 1. NMR Data of Compound **1** Recorded in CD_3OD (500 MHz)

position	DEPT	^{13}C	^{1}H
1	CH_2	39.6	0.96 m/1.63 m
2	CH_2	26.5	1.74 m/1.81 m
3	CH	84.3	3.31 dd (11.1, 4.5)
4	C	40.2	
5	CH	57.0	0.74 m
6	CH_2	19.3	1.40 m/1.56 m
7	CH_2	30.8	1.65 m/1.31 m
8	C	40.7	
9	CH	48.0	1.56 m
10	C	37.8	
11	CH_2	24.0	1.92 m/2.05 m
12	CH	123.8	5.34 m
13	C	144.9	
14	C	42.8	
15	CH_2	28.9	1.74 m/1.06 m
16	CH_2	24.6	2.09 ddd (13, 12, 5)/1.74 ddd (12, 3, 5)
17	C	47.4	
18	CH	43.9	2.81 dd (12.0, 4.0)
19	CH_2	43.3	2.09 dd (12, 5)/2.6 dd (12, 11)
20	C	44.9	
21	CH_2	31.3	1.24 m/1.34 m
22	CH_2	34.3	1.63 m/1.56 m
23	CH_3	26.3	0.94 s
24	CH_3	16.0	0.83 s
25	CH_3	17.0	1.01 s
26	CH_3	17.8	0.82 s
27	CH_3	26.3	1.20 s
28	C	178.7	
29	C	177.5	
30	CH_3	28.6	1.17 s
OCH_3	CH_3	52.4	3.72 s

spectrum showed absorptions at 3350 (OH) and 1700 (C=O of COOH) cm^{-1}. The FABMS of **1** showed the $[M - H]^-$ ion at *m/z* 955 and prominent fragments at *m/z* 793 $[(M - H) - 162]^-$ (cleavage of one hexose unit), *m/z* 631 $[(M - H) - (162 \times 2)]^-$ (cleavage of two hexose units), and *m/z* 499 $[(M - H) - (162 \times 2) - 132]^-$ (due to the loss of two hexose units and one pentose moiety, respectively). The ^{13}C NMR spectrum showed 48 signals, of which 31 were assigned to a triterpenoid moiety and 17 to the saccharide portion. In the ^{1}H NMR spectrum of **1**, the sugar portion exhibited three anomeric proton signals (δ 5.34, d, $J = 8.1$ Hz; 4.57, d, $J = 7.3$ Hz; and 4.37, d, $J = 8.4$ Hz) (Table 2). The other sugar signals overlapped in the region δ 3.31−3.99. The structures of the oligosaccharide moieties were deduced using COSY and HMQC experiments. COSY experiments allowed the sequential assignments of all of the proton resonances to the individual monosaccharides, as reported in Table 2. The chemical shifts, the multiplicity of the signals, the absolute values of the coupling constants, and their magnitude in the ^{1}H NMR spectrum as well as ^{13}C NMR data (Table 2) indicated the β-configuration at the anomeric position for the glucopyranoses ($J = 8.1$ and 7.3 Hz) and the α-configuration at the anomeric position for the arabinopyranosyl unit ($J = 8.4$ Hz). HMQC experiments, which correlated all the proton resonances with those of each corresponding carbon, allowed the assignments of the interglycosidic linkages of **1** by comparison of the observed carbon chemical shifts with those of the corresponding free sugars. The absence of any ^{13}C NMR glycosidation shift for one β-D-glucopyranosyl and one α-L-arabinopyranosyl moiety suggested that these sugars were terminal units. A glycosidation shift was observed for C-3_{ara} (83.6 ppm). Chemical shifts of H-1_{Glc} (δ 5.34) and C-1_{Glc} (95.7 ppm) indicated that this sugar unit was involved in an ester linkage with the C-28 carboxylic group.[23,24] The position of the sugar residues in **1** were defined unambiguously by the HMBC[30] experiment. A cross-peak due to long-range correlations between C-3 (84.3 ppm) of the aglycon and H-1_{Ara} (δ 4.37) indicated that arabinose was the residue linked to C-3 of the aglycon; a cross-peak between C-3_{ara} (83.6 ppm) and H-1 of the glucose (δ 4.57) indicated that glucose was the second unit of the disaccharide chain at C-3 of the aglycon. Similarly, a cross-peak between H-1 of glucose (δ 5.34) and the ^{13}C NMR resonance of the aglycon carbonyl group (178.7 ppm) provided definitive proof for an ester linkage between one glucose unit and the aglycon. The HMBC cross-peak between the anomeric glucose proton and C-28 was in full agreement with the results of alkaline hydrolysis giving a new monodesmoside (**1a**), whose ^{1}H and ^{13}C NMR spectra showed no signals ascribable to a *O*-β-D-glucopyranoside in an ester linkage, but exhibited signals ascribable to serjanic acid 3-*O*-[β-D-glucopyranosyl-(1″→3′)-α-L-arabinopyranosyl]. On the basis of all of this evidence, compound **1** was identified as serjanic acid 3-*O*-[β-D-glucopyranosyl-(1″→3′)-α-L-arabinopyranosyl]-28-*O*-β-D-glucopyranoside.

Table 2. NMR Spectral Data of Sugar Moieties of Compound **1** Recorded in CD_3OD

position	^{13}C	^{1}H	position	^{13}C	^{1}H
β-D-Glc			β-D-Glc		
1	95.7	5.34 d (8.1)	1″	106.1	4.57 d (7.3)
2	75.3	3.31 dd (9.5, 8.1)	2″	73.9	3.37 dd (9.0, 7.5)
3	78.7	3.47 t (9.5)	3″	77.9	3.43 t (9.0)
4	71.1	3.31 t (9.5)	4″	71.2	3.31 t (9.0)
5	78.3	3.43 m	5″	77.7	3.41 m
6	62.4	3.67 dd (12.0, 5.0)	6″	62.3	3.64 dd (12.0, 5.0)
		3.72 dd (12.0, 5.0)			3.83 dd (12.0, 5.0)
α-L-Ara					
1′	105.5	4.37 d (8.4)			
2′	72.1	3.86 dd (5.4, 8.4)			
3′	83.6	3.83 t			
4′	69.5	3.99 m			
5′	66.9	3.85 dd (12, 3.5)			
		3.53 dd (12, 3.5)			

Experimental Section

General Experimental Procedures. Optical rotations were determined on a Perkin-Elmer 192 polarimeter equipped with a sodium lamp (589 nm) and a 10 mm microcell. The FT-IR spectra were obtained on a Bruker IFS-48 spectrophotometer using a KBr matrix. The NMR spectra were obtained in CD_3OD with a Bruker AMX 500 spectrometer. The DEPT experiments were performed with a pulse of 135° to obtain positive signals for CH and CH and negative signals for CH_2; an average CH coupling constant of 135 Hz was assumed. ^{1}H detected heteronuclear multiple quantum coherence (HMQC) experiments were performed according to the procedure of Martin and Crouch,[30] using an initial BIRD pulse to suppress ^{1}H resonances not coupled to ^{13}C and a GARP sequence for ^{13}C decoupling during data acquisition. The spectral width in the ^{1}H dimension was 2994.05 Hz; 256 experiments of 240 scans each (relaxation delay = 1.5 s, delay after BIRD pulse = 0.4 s, fixed delay $t_1 = 3.3$ ms) were acquired in 1 K points. A sine square function was applied in the t_2 dimension, and a trapezoidal window was applied in the t_1 dimension ($TM_1 = 0.03$ Hz, $TM_2 = 0.6$ Hz) before Fourier transformation (digital resolution in F_2 dimension = 2.994 Hz/point). ^{1}H detected heteronuclear multiple bond correlation (HMBC) spectroscopy was performed according to the methods of Bax et al.[31] and

Martin and Crouch.[30] The data processing was identical to that used for the HMQC experiment, and the final digital resolution was 2.25 Hz/pt. FABMS (recorded in a glycerol matrix) were measured on a Prospec Fisons mass spectrometer, and HR-FABMS were recorded in a glycerol matrix on a VG AUTOSPEC instrument. GC−MS was run using a Hewlett-Packard 5890 gas chromatograph equipped with an HP-5 column (25 m × 0.2 mm i.d., 0.33 μm film), fitted with an HP 5970B mass detector and an HP 59970 MS Chemstation. HPLC separations were performed on a Hewlett-Packard HP 1050 series apparatus with a Varian RI-4 refractive index detector, equipped with Waters μ-Bondapack C_{18} column (7.8 × 300 mm). DCCC (droplet counter-current chromatography) separation was performed on a Buchi apparatus equipped with 300 tubes.

Plant Material. The plant material was collected in Peru in April 1999 and identified by Dr. S. E. Jacobsen of the International Potato Centre (CIP), Lima, Peru. A voucher sample has been deposited in the Herbarium Neapolitanum of the Dipartimento di Biologia Vegetale Università degli Studi "Federico II" of Naples. The collection number is NAP # A. C. 002.

Extraction and Isolation. The whole flour from the seeds (709 g) was extracted with MeOH. The MeOH extract (49.03 g) was partitioned between *n*-BuOH and H_2O. The butanol extract (26.2 g) was evaporated and defatted with $CHCl_3$. The residue fraction (10 g) was chromatographed on a Sephadex LH-20 column (100 × 5 cm), with MeOH as eluent. Fractions (9 mL) were collected and checked by TLC [Si gel plates in *n*-BuOH−HOAc−H_2O (60:15:25)]. Fractions 23−57 (3214 mg) containing the crude glycosidic mixture were further separated by DCCC using *n*-BuOH−Me_2CO−H_2O (60:12:28) as stationary phase and *n*-BuOH−Me_2CO−H_2O (14:12:74) as descending phase. DCCC fractions 48−296 (527 mg) containing the crude glycosidic mixture were chromatographed by reversed-phase HPLC with MeOH−H_2O at a flow rate of 2.2 mL/min to yield seven pure compounds.

Serjanic acid 3-*O*-[*β*-D-glucopyranosyl-(1″→3′)-α-L-arabinopyranosyl]-28-*O*-*β*-D-glucopyranoside (1): white powder (40.1 mg); $[\alpha]^{25}_D$ +10° (*c* 0.003 in MeOH); IR (KBr) ν_{max} 3350, 1700 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1 and 2; negative-ion FABMS *m/z* 955 [M − H]$^-$, 793, 631, 499; negative-ion HRFABMS *m/z* 956.4986, calcd for $C_{48}H_{76}O_{19}$ 957.1056.

Phytolaccagenic acid 3-*O*-[α-L-arabinopyranosyl-(1″→3′)-*β*-D-glucuronopyranosyl]-28-*O*-*β*-D-glucopyranoside: white powder (6.2 mg); $[\alpha]^{25}_D$ +10.8° (*c* 0.003 in MeOH);[23] HRFABMS *m/z* 985.4711, calcd for $C_{48}H_{74}O_{21}$ 985.4723.

Oleanolic acid 3-*O*-[*β*-D-glucopyranosyl-(1″→3′)-α-L-arabinopyranosyl]-28-*O*-*β*-D-glucopyranoside: white powder (1.0 mg); $[\alpha]^{25}_D$ +3° (*c* 0.002 in MeOH);[23] HRFABMS *m/z* 911.5094, calcd for $C_{47}H_{76}O_{17}$ 911.5082.

Hederagenin 3-*O*-[*β*-D-glucopyranosyl-(1″→3′)-α-L-arabinopyranosyl]-28-*O*-*β*-D-glucopyranoside: white powder (71.5 mg); $[\alpha]^{25}_D$ +31.1° (*c* 1.00 in MeOH);[24] HRFABMS *m/z* 927.50317, calcd for $C_{47}H_{76}O_{18}$ 928.09554.

Phytolaccagenic acid 3-*O*-[*β*-D-glucopyranosyl-(1″→3′)-α-L-arabinopyranosyl]-28-*O*-*β*-D-glucopyranoside: white powder (27.0 mg); $[\alpha]^{25}_D$ +5.8° (*c* 0.005 in MeOH);[23] HRFABMS *m/z* 971.4945, calcd for $C_{48}H_{76}O_{20}$ 971.4930.

Oleanolic acid 3-*O*-[*β*-D-glucuronopyranosyl]-28-*O*-*β*-D-glucopyranoside: white powder (28.1 mg); $[\alpha]^{25}_D$ +6.9° (*c* 0.005 in MeOH);[25] HRFABMS *m/z* 793.44526, calcd for $C_{47}H_{76}O_{17}$ 793.96504.

Oleanolic acid 3-*O*-[α-L-arabinopyranosyl-(1″→3′)-*β*-D-glucuronopyranosyl]-28-*O*-*β*-D-glucopyranoside: white powder (21.2 mg); $[\alpha]^{25}_D$ +2° (*c* 0.002 in MeOH);[33] HRFABMS *m/z* 925.4886, calcd for $C_{47}H_{74}O_{18}$ 925.4875.

Acid Hydrolysis of Compound 1 (Monosaccharide Composition). A solution of **1** (1 mg) in 10% H_2SO_4−EtOH (1:1, 3.5 mL) was refluxed for 4 h. The reaction mixture was diluted with H_2O and then extracted with Et_2O. The Et_2O layer was dried with anhydrous Na_2SO_4 and evaporated to dryness. The H_2O layer was neutralized with Amberlite MB-3 ion-exchange resin and evaporated to dryness. The resulting monosaccharides were reacted with TRISIL-Z (Pierce, Rockford, IL) and analyzed by GC−MS obtaining glucose and arabinose in the ratio 2:1. Retention times were identical to those of the authentic trimethylsilylated sugars.

Alkaline Hydrolysis. Glycoside **1** was heated in 0.5 N aqueous KOH (1 mL) at 110 °C in a reaction vial for 2 h. The reaction mixture was adjusted to pH 7 with HCl and then extracted with *n*-BuOH and analyzed by NMR data, obtaining serjanic acid 3-*O*-*β*-D-glucopyranosyl-(1″→3′)-α-L-arabinopyranoside (**1a**).

Serjanic acid 3-*O*-*β*-D-glucopyranosyl-(1″→3′)-α-L-arabinopyranoside (1a): IR (KBr) ν_{max} 3350, 1700 cm^{-1}; ^1H NMR (CD_3OD) δ 5.34 (1H, t, J = 3.4 Hz, H-12), 2.81 (1H, dd, J = 14.3, 3.7 Hz, H-18), 0.94 (3H, s, H-23), 0.83 (3H, s, H-24), 1.01 (3H, s, H-25), 0.82 (3H, s, H-26), 1.20 (3H, s, H-27), 1.17 (3H, s, H-30), 3.72 (3H, s, OCH_3), 4.37 (1H, d, J = 8.4 Hz, H-1′), 4.57 (1H, d, J = 7.5 Hz, H-1″); ^{13}C NMR (CD_3OD, 500 MHz) δ 39.6 (C-1), 26.5 (C-2), 84.3 (C-3), 40.2 (C-4), 57.0 (C-5), 19.3 (C-6), 30.8 (C-7), 40.7 (C-8), 48.0 (C-9), 37.8 (C-10), 24.0 (C-11), 123.8 (C-12), 144.9 (C-13), 42.8 (C-14), 28.9 (C-15), 24.6 (C-16), 47.4 (C-17), 43.9 (C-18), 43.3 (C-19), 44.9 (C-20), 31.3 (C-21), 34.3 (C-22), 26.3 (C-23), 16.0 (C-24), 17.0 (C-25), 17.8 (C-26), 26.3 (C-27), 182.6 (C-28), 177.5 (C-29), 28.6 (C-30), 52.4 (OCH_3), 105.5 (C-1′), 72.1 (C-2′), 83.6 (C-3′), 69.5 (C-4′), 66.9 (C-5′), 106.1 (C-1″), 73.9 (C-2″), 77.9 (C-3″), 71.2 (C-4″), 77.7 (C-5″), 62.3 (C-6″); negative-ion FABMS *m/z* 793 [M − H]$^-$, 631, 499; negative-ion HRFABMS *m/z* 793.95707, calcd for $C_{42}H_{65}O_{14}$ 793.43743.

Acknowledgment. The assistance of Dr. S. E. Jacobsen of the International Potato Centre (CIP), Lima, Peru, is gratefully appreciated. The NMR and GC−MS data were obtained at the "Centro di Ricerca Interdipartimentale di Analisi Strumentale", and FABMS were performed at the "Servizio di Spettrometria di Massa" of the University "Federico II" Napoli.

References and Notes

(1) National Research Council. *Lost Crops of the Incas*: *Little Known Plants of the Andes with Promise for World-wide Cultivation*; National Academy Press: Washington, DC, 1989; pp 149−162.
(2) Schlick, G.; Bubenheim, D. L. *NASA Technol. Paper* **1993**, 3422−3424.
(3) Koziol, M. J. *J. Food Comp. Anal.* **1992**, *5*, 35−68.
(4) Oda, K.; Matsuda, H.; Murakami T.; Katayama, S.; Ohgitani T.; Yoshikawa, M. *J. Biol. Chem.* **2000**, *381*, 67−74.
(5) Orjih, A. U. *Lancet* **1994**, *343*, 295−296.
(6) Paz-Aliaga, B.; Fitzpatrick, D. W. *Bol. Soc. Quim. Peru* **1992**, *58*, 69−74.
(7) Gnabre, J. N.; Halonen, M. J.; Martin, D. G.; Pinnas J. L. *Int. J. Immunopharmacol.* **1994**, *16*, 641−650.
(8) Lee, K. T.; Sohn, I. C.; Kim, D. H.; Choi, J. W.; Kwon, S. H.; Park, H. J. *Arch. Pharmacol. Res.* **2000**, *23*, 461−466.
(9) Yoshikawa, M.; Murakami, T.; Matsuda, H.; Ueno, T.; Kadoya, M.; Yamahara, J.; Murakami, N. *Chem. Pharm. Bull.* **1996**, *44*, 1305−1313.
(10) Kim, H. P. *Arch. Pharmacol. Res.* **1999**, *22*, 313−316.
(11) Ju, D. W.; Zheng, Q. Y.; Cao, X. T.; Fang, J.; Wang, H. B. *Pharmacology* **1998**, *56*, 187−195.
(12) Charlet, S.; Gillet, F.; Villarreal, M. L.; Barbotin, J. N.; Fliniaux, M. A.; Nava-Saucedo, J. E. *Plant Physiol. Biochem.* **2000**, *38*, 875−880.
(13) Villarreal, M. L.; Nicasio, P.; Rojas, G.; Alvarez, L.; Quintero, R. *Adv. Exp. Med. Biol.* **1999**, *464*, 221−233.
(14) Zehavi, U.; Polacheck, I. *Adv. Exp. Med. Biol.* **1996**, *404*, 535−546.
(15) Simoes, C. M. O.; Amoros, M.; Girre, L. *Phytother. Res.* **1999**, *13*, 323−328.
(16) Park, H. J.; Kwon, S. H.; Lee, J. H.; Lee, K. H.; Miyamoto, K. I.; Lee, K. T. *Planta Med.* **2001**, *67*, 118−121.
(17) Wang, H. X.; Ng, T. B. *Life Sci.* **1999**, *65*, 2663−2670.
(18) Venugopalan, C.; Srivastava, K. N. *J. Food Sci. Technol.* **1996**, *33*, 389−392.
(19) Plohmann, B.; Bader, G.; Hiller, K.; Franz, G. *Pharmazie* **1997**, *52*, 953−957.
(20) Bandara, B., M., R.; Jayasinghe, U. L. B.; Karunaratne, V.; Wannigama, G. P.; Bokel, M.; Kraus, W.; Sotheeswaran, S. *Planta Med.* **1990**, *56*, 290−292.
(21) Mitsue, H.; Motidome, M.; Gottlieb, O. R. *Phytochemistry* **1988**, *27*, 2291−2296.
(22) Morales, M., A. *Rev. Latinoam. Quim.* **1978**, *9*, 94−95.
(23) Dini, I.; Schettino, O.; Simioli, T.; Dini, A. *J. Agric. Food Chem.* **2001**, *49*, 741−746.

(24) Dini, I.; Tenore, G. C.; Schettino, O.; Dini, *J. Agric. Food Chem*. **2001**, *49*, 3976−3981.
(25) Mizui, F.; Kasai, R.; Otani, K.; Tanaka, O. *Chem. Pharm. Bull.* **1990**, *38*, 375−377.
(26) Rastrelli, L.; De Simone, F.; Schettino, O.; Dini, A. *J. Agric. Food Chem*. **1996**, *44*, 3528−3533.
(27) Woldemichael, G., M.; Wink, M. *J. Agric. Food Chem*. **2001**, *49*, 2327−2332.
(28) Tamura, Y.; Mizutani, K.; Ikeda, T.; Ohtani, K.; Kasai, R.; Yamasaki, K.; Tanaka, O. *Nat. Med*. **2001**, *55*, 11−16.
(29) Quetin-Leclercq, J.; Elias, R.; Balansard, G.; Bassleer, R.; Angenot, L. *Planta Med*. **1992**, *58*, 279−281.
(30) Bodenhausen, G.; Ruben, D. *J. Chem. Phys. Lett*. 1**980**, *69*, 185−186.
(31) Martin, G. E.; Crouch, R. C. *J. Nat. Prod*. **1991**, *54*, 1−70.
(32) Bax, A.; Aszavalos, A.; Dinya, Z.; Sudo, K. *J. Am. Chem. Soc.* **1986**, *108*, 8056−8057.
(33) Iwamoto, M.; Okabe, H.; Yamauchi, T. *Chem. Pharm. Bull.* **1985**, *33*, 1−7.

NP010625Q