ISOLATION AND STRUCTURE ELUCIDATION OF THE CUSHION PLANT *POTENTILLA ARTICULATA*

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ABSTRACT

Methanol extract of the whole plant of *P. articulata* Franch was fractionated by using open column silica gel chromatography following identification, resulted in the isolation of known phytoceramides, N-(2'-hydroxy-acyl)-2-amino-1,3,4trihydroxy-8-octadecene) (1), which also isolated from *Urtioca dioca* and *Thylaco-spermum caespitosum,* and two known steroids, identified as β-sitosterol (2), 3β-O-β-D-gluco-pyranosylsitosterol (3), which have also been isolated from *Prunella vulgaris* L. var. lilacina (Labiatae). Triterpenoic acids were elucidated as 2β,19α-dihydroxyursolic acid (4), a mixture of 2α-monohydroxyursolic acid (5) and 2α-mono-hydroxyoleanolic acid (6), also found in *Geurn japonicum* Thunberg, 19a-monohydro-xyursolic acid (7), a mixture ursolic acid (8) and oleanolic acid (9), which were also obtained in *Isodon japonicus* Hara. Their structure identifi-cations were based on chemical and spectroscopic methods.

INTISARI

Ekstrak metanol dari tanaman *P. articulata* Franch difraksinasi dengan menggunakan kolom terbuka silika gel kromatografi kemudian identifikasi, didapatkan senyawa-senyawa yang telah diketrahui yaitu phytoceramides N-(2' hydroxy-acyl)-2-amino-1,3,4-trihydroxy-8-octadecene (1), yang juga telah diisolasi dari *Urtioca dioca* and *Thylacospermurn caespitosurn* dan dua senyawa steroid, diidenti-fikasi sebagai β-sitosterol (2), 3β-O-β-D-gluco-pyranosylsitosterol (3), yang telah diisolasi dari *Prunella vulgaris* L, var. lilacina (Labiatae). Beberapa senyawa asam triterpenoat juga diisolasi yang dielusidasi sebagai 2β,19α-dihydroxyursolic acid (4), campuran senyawa 2α-monohydro-xyursolic acid (5) dan 2α-monohydroxyoleanolic acid (6), yang juga ditemukam dalam *Geurn japonicum* Thunberg, dan 19α-monohydro-xyursolic acid (7), campuran senyawa asam ursolat (8) dan asam oleanolat (9), yang juga didapatkan dalam *Isodon japonicus* . Senyawa-senyawa tersebut diidentifikasi berdasarkan metoda kimia dan spektroskopi.

INTRODUCTION

During the course of our studies on the chemical constituents of medicinal plants from some countries, a number of biologically active compounds, primarily flavonoids and new triterpenoid saponins have been isolated [6]. In continuing the studies on the new biologically active compounds of some plants, the cushion plants, P. *articulata* Franch belongs to the family Rosaceae were collected from strict climate zone in Tibet at a mountain about 5,000 m height. A number of plant species of Rosaceae [1, 2], are used as folk medicines for variety of diseases in diverse areas of the world. Among them, *Geurn japonicurn* Thunberg is a perennial herb and the flowering plant has been used in Japan as diuretic, had been isolated a mixture of triterpenoic acids, 2α-19α-dihydroxyursolic (4) 2αmonohydroxyur-solic acid (5), 2α-monohydro-xyoleanolic acid (6), and 2α,3β,19α, 23-tetrahydroxyurs-12-en-28-oic acid 28-O-β-D-glucopyranoside (Niga ichigoside F1). From *Potentilla kleiniana* Wight et Arnott were isolated tannins as potentillin, agrimoniin and pedunculagin, which is also used similarly as a folk medicine in Japan [1]. The authors have taken interest in cushion plant of *P. articulata,* because no chemical studies have been reported. The present paper describes the isolation and structure elucidation of the methanol extract of P. *articulata.* The whole plant extract was isolated by using column chromatography and the structure of the constituents were elucidated on the basis of IR, MS and NMR analysis.

EXPERIMENTAL

General Methods.

All melting points were determined on a Yanaco micro melting point apparatus and are uncorrected, optical rotations ($[\alpha]_D$)were measured in CHC13 solution on Jasco DIP-370 Digital Polarimeter. IR spectra were recorded with Jasco IR-100 spectrometer in Nujol or CHC13; MS were obtained with JEOL AX500 mass spectrophotorneter, using a direct inlet system. ^1H and ^{13}C NMR spectra (400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR) were recorded on JEOL GX400 spectrometer in CDC13 at 40 °C with tetramethylsilane (TMS) as internal standard for ^1H NMR and solvent as internal reference for ^{13}C NMR (at δ 77.03); chemical shift values (δ) are given in ppm; the abbrviations s = singlet, d = doublet, t= triplet, q = quartet, m = multiplet, dd = double doublet, and ddd = double double doublet are used throught. Fractionation and purification of methanol extract with using open column silica gelchromatography were monitored with TLC. The TLC identificatons were carried out on Merck silica gel GF254 plates and the spots were examined under UV light, and visualized with anisaldehyde. Column chromatography was conducted on silica gel, Fuji Silysia BW820 MH.

Plant material.

The sample of cushion plants, *Potenalla articulata* Franch with Chinese name "Kan bin guan wei lin chai", were collected in 1991 in Tibet.

Extraction and isolation of compounds 1 - 9.

The dried whole plant of P. *articulata* (163 g) was extracted in MeOH (2 x 1.2 litres) to give MeOH extract about 2 g. It was directly chromatographed on silica gel column using CH2C12-MeOH gradient elutions to yield seven fractions. Fraction 3 (40 mg) was recrystallized to give compound 2 (30 mg). Three of these fractions 4 (50 mg), 5 (260 mg) and 6 (80 mg) was acetylated by acetic anhydride pyridine, followed to work up in the usual manner afforded compound 7a (6 mg) and a mixture of 8a and 9a (8 mg) from fraction 4; compound la (10 mg), 4a (5 mg) and a mixture of compounds 5a and 6a (11 mg) from &action 5 and 3a (60 mg) &om &action 6, respectively.

Hydrolysis Compound of la.

Compound la was treated by 3 % H2SO4 in MeOH for three days at 37 °C. After neutralization with diluted NaHCO3 solution and extraction with ethyl acetate, the medhylates were investigated by EIMS to give three molecular peaks M+ at m/z 426, 412 and 398.

Physicochemical data of compounds 1a - 9a.

Compound la is a mixture of phyto-ceramides, amorphous powder; mp. 40-43 °C; [a] $[\alpha]^{24}{}_D$ +0.32 (c 0.1; CHC13); IR (Nujol) ν cm^{-1} : 3360, 1750, 1660, 1540, 1220, 720. FAB-MS m/z: 877, 863, 849 (each, M+). ^1H NMR (CDC13): δ 6.55 (1H, NH, d, J = 9.2 Hz), 5.37 (H-8, =CH, ddd, J = 11.6, 6.1, 3.5 Hz), 5.34 (H-9, =CH, ddd, J = 11.6, 5.8, 3.5 Hz), 5.05 (1H, H-3, dd, J = 7.3, 3.7 Hz), 4.96 (H-4,dt,J=9.8,6.4Hz),4.3(H-la, dd, J=11.9, 5.2Hz), 4.0 (H-lb,dd,J=ll.9, 3.4 Hz), 5.08 (H-2', dd. J = 7.3, 5.2 Hz), 4.44 (1H, ddd, J = 6.1 Hz), four acetyl groups at δ 2.0, 2.03, 2.06, 2.13 (each, s, 3H), 1.97 (CH2-10, m), 1.82, (CH2-7), 1.66 and 1.63 (each, 1H, CH2-5, m), 1.26 (bs, CH2), 0.88 (2xCH3, t, J = 6.7 Hz),. ^{13}C NMR: δ14.1(q, 2xCH3), 29.7 (t, (CH2)n), 131.1 (d, C-8), 129.3 (d, C-9), 28.5 (CH2-5), 32.6 (CH2-6), 24.9 (CH2-7, 62.4 (t, C-1), 72.5 (d, C-3), 72.6 (d, C-4), 74.1 (d, C-2'), 48 (d, C-2, CH-NH-), 171.2, 170.2, 170.0, 169.9, 169.9 (each, s), 32.6 (CH2-7, t), 31.9 (CH2-6, t), 20.99, 20.84, 20.70 and 20.71 (each, CH3, q).

Compound 2, β-sitosterol. Colorless needles, mp 130-133 °C; IR (Nujol) ν cm^{-1}: 3400 (OH), 1660, 1100, 1050, 800. EIMS mJz: 414 (M+, base peak), 396, 303, 273, 255. ^1H NMR (CDC13): δ 3.52 (H-3, m), 5.34 (1H, H-6, dd, J = 5.2 Hz), 0.68 (CH3-18, s), 1.05 (CH3-l9, s), 0.92 (CH3-21, d, J = 6.4 Hz), 0.82 (CH326, d, J = 10 Hz), 0.85 (CH3-27, d, J = 7.6 Hz), 0.80 (CH3-29, t, J = 7.0 Hz).

Compound 3a, β-sitosterolglucosyltetra-acetate; amorphous powder; mp. 163-165°C; [α] $^{24\ D}$ -27.43 (c 0.11; CHC13); IR (Nujol) ν cm^{-1}: 1755, 1220, 1160, 1100, 1050. EIMS mSz: 744 (M+), 685, 644, 601, 413, 396, 331. H NMR (CDC13): o 3.48 (1H, H-3a, tt, J = 9.4, 5.8 Hz), 5.37 (1H, H-6, dd, J = 8.9, 3.7 Hz), 0.68 (3H, s), 0.99 (3H, s), 0.93 (3H, d, J = 6.4 Hz), 0.84 (3H,

d, J = 7.1 Hz), 0.81 (3H,d,J= 7.1 Hz),0.85(3H,t,J= 7.6Hz),4.59(H-1',4.59(d,J =7.9Hz),4.96 (H-2', dd, J = 7.9, 9.5 Hz), 5.20 (H-3', t, J = 9.5 Hz), 5.08 (H-4', t, J = 9.5 Hz), 3.68 (H-5', dd., J = 9.5, 5.2, 2.8 Hz), 4.13 aH-6'a, dd, J = 12.5, 2.8 Hz), 4.25 (H6'b, dd, J = 12.5, 5.2 Hz). ^{13}C NMR: δ 80.1 (d, C-3), 140.5 (s, C-5), 122.1 (d, C6), 11.9 (CH3-18), 19.4 (CH3-19), 18.8 (CH3-21), 19.8 (CH3-26), 19.1 (CH3-27), 12.0 (CH3-29), 99.7 (C-1', anomeric carbon), 71.7 (C-2'), 73.1 (C-3'), 68.8 (C4'), 71.8 (C-5'), 61.3 (C-6'), 170.6, 170.3, 169.4, 169.3 (each, -COG), and 20.7, 20.7, 20.6, 20.6 (each, CH3COO-).

Compound 4a, 2α,19α-diacetylursolic acid; white powder, mp 90-92°C; [α] $^{24}{}_{D}$ -15.78 (c 0.13; CHC13); IR (CHCI3) ν cm^{-1}: 3500, 1735, 1740, 1450, 1360, 1240, 1030. EIMS (%) m/z: 572 (M+, 8), 554 (20), 526 (30), 454 (12), 264 (20), 246 (32).

Compounds 5a and 6a, 2α-acetylursolic acid and 2a-acetyloleanolic acid. Whitepowder, mp 181-183°C; [α] $^{24}{}_{D}$ +14.79 (c 0.13; CHC13); IR (CHC13) ν cm^{-1}: 1740, 1700, 1370, 1240, 1030. EIMS (%): mSz 556 (M+, 2.6), 541 (1), 510 (3.6), 248 (base peak), 203 (45).

Compound 7a, 3β-acetyl,19α-hydroxyursolic acid; white powder, mp 240 243°C; [α] $^{24}{}_{D}$ +39.18 (c 0.1; CHC13); IR (CHC13) ν cm^{-1}: 3500, 1720, 1700, 1250. EIMS (%) m!z: 514 (M+, 5), 468 (23), 454 (18), 439 (6), 396 (10), 264 (22).

Compounds 8a and 9a, 3β-acetylursolic acid and 3,β-acetyloleanolic acid; white powder, mp 175-178°, [α] $^{24}{}_{D}$ +78.79 (c 0.25; CHCl3); IR (CHCl3) ν cm^{-1}: 1720, 1250. EIMS (%) m/z: 498 (M+, 3), 438 (4.5), 248 (base peak), 203 (45) (Figure 1).

RESULTS AND DISCUSSION

Compound 1.

The Mixture of phytoceramides (1) obtained as amorphous white powder, was acetylated to give tetra acetyl derivative la, mp. 40-43°C. Its IR spectrum exhibited absorption based on NH of amide at 3360 cm^{-1}, ester at 1750 cm^{-1}, amide carbonyl at 1700 cm^{-1} . From the EIMS of la was observed three molecular peaks (M+) at m/z 877, 863, and 849 to be a mixture of three components.

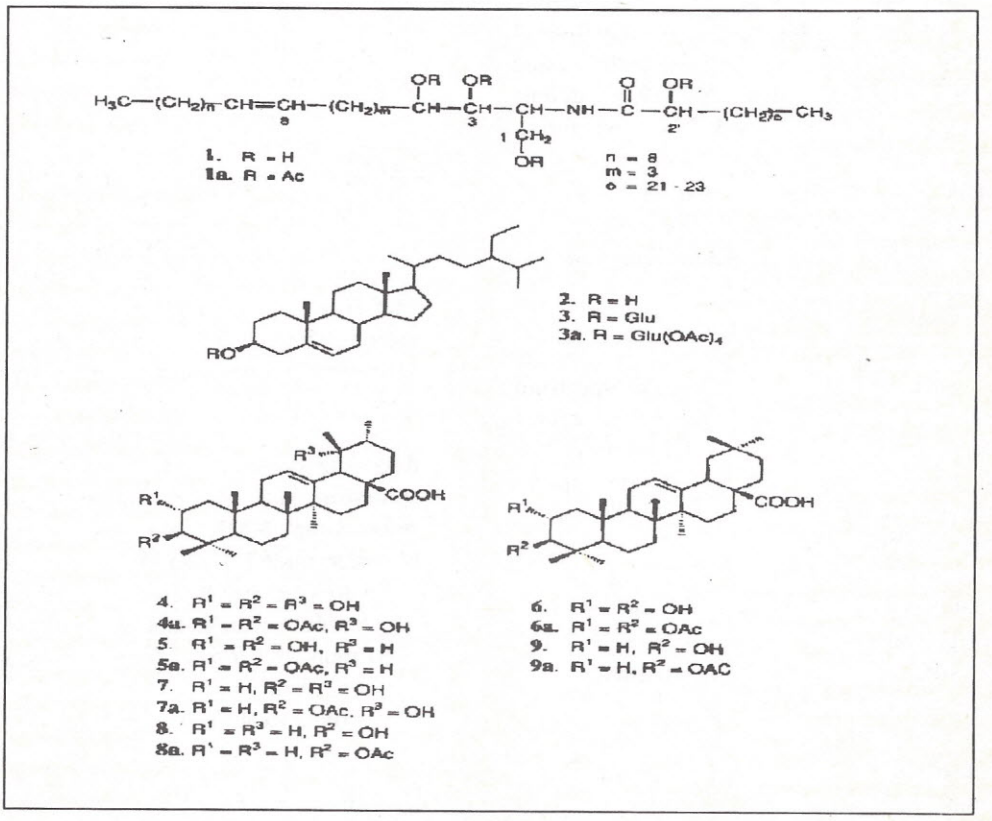
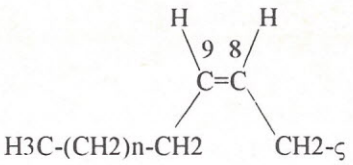


Figure 1. The structure of chemical constituents of *P. articulata* F.

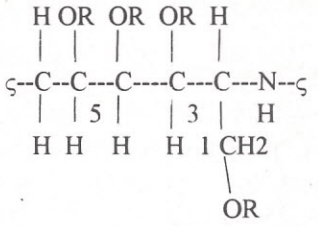
The ^{1}H NMR spectrum of la, revealed the signals of two methyls at δ 0.88 (6H, t, J = 6.7 Hz), cis olefenic protons at δ 5.37 (ddd, J = 11.6, 6.1, 3.5 Hz) and 5.34 (ddd, J = 11.6, 6.1, 3.5 Hz), and (CH2)n group at δ 1.26 (bs). The four acetyl signals at δ 2.0, 2.03, 2.06, and 2.13 indicated the presence of four hydroxyl groups, corresponding to signals of oxymethylene at δ 4.3 (dd, J = 11.9, 5.2 Hz) and 4.0 (dd, J = 11.9, 3.4 Hz) for CH2- 1, three oxymethines at δ 5.05 (dd, J = 7.3, 3.7 Hz) for CH-3, 4.96 (dt, J = 9.8, 6.4 Hz) for CH-4, and 5.08 (dd, J = 7.3, 5.2 Hz) for CH-2'. The methylene protons for CH2-5 and CH2-3' appeared at δ 1.63 and 1.66, and 1.82 (each, m), the methine proton of CH-2 appeared at δ 4.44 (m). an additional the most downfield signal was observed at δ 6.55 (d, J = 9.2 Hz) corresponding to the presence of NH of amide group.

The ^{13}C NMR spectrum of la also revealed the signals of two methyls at δ 14.1 (q, 2xCH3), an unsaturated carbon at δ 131.2 (d, C-8) and 129.3 (d, C-9), and (CH2)n group at δ 29.7 (t). The four acetate methyls were indicated at δ 20.70, 20.71, 20.84, and 20.99 (each, q), and five carbonyl groups at δ 169.9 (double intensity), 170.0, 170.2, and 171.2 (each, s). Further, oxymethylene carbon at δ 62.4 (t, C-1), methylene at δ 28.5 (t, C-5), and 31.9 (t, C-3'), three oxymethine carbons at δ 72.5 (d, C-3), 72.6 (d, C-4) and 74.1 (d, C-2'), and also methine at δ 48.0 (d, C-2).

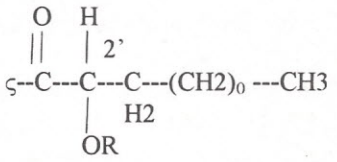
The 2D ^{1}H- ^{1}H RCOSY spectrum indicated the presence of three partial structures of A, B and C (Scheme 1). In order to clarify the partial structures of la, spin decoupling experiments were examined. The irradiation of the some protons also supported the presence of partial structures A and B. Irradiation of the proton at δ 4.44, clearly indicated the coupling with the protons at δ 4.3, 4.0, 5.05 and 6.55 (the signals were changed). The irradiation of the proton at δ 4.96 showed that it was coupled with δ 5.05, 1.66, and 1.63. The irradiation of the proton at δ 1.82 showed that it was coupled with the proton at δ 5.08.



(A)



(B)



(C)

Figure 2. Partial structure of compound 1a, derived from ^{1}H-^{1}H-RCOSY

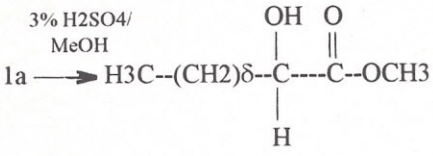
In view of the above spectral evidence, the comparison of ^{1}H and ^{13}C NMR spectral data with those of similar ceramide [3, 4], established structure la. Methanolysis (methylation and hydrolysis) of this compounds with H2SO4 in MeOH provided a mixture of 2-hydroxy methyl ester, which had been identified by the characteristic mass spectra, M at m/z 426, 412, and 398 (Figure 3).

From these results and the literature data, the double bond must be located at the spingosine side [5]. Combining this mass spectral information, NMR data and the other similar phytoceramide [3], deduced the structure of 1, N-(2'-hydroxyacyl)-2-amino-1,3,4-trihydroxy-8-octadecene), which had already been isolated from roots of *Ultioca dioca* (Urticaceae) [4] and cushion plant of *Thylacospermum caespitosum* (Caryophyllaceae) [6].

Compounds 2 and 3.

The ^{1}H NMR spectrum of compound 2 indicated the presence of two tertiary methyls at δ

0.68 and 1.0, three secondary methyls at δ 0.92, 0.83 and 0.81 (each, d, J = 6.4 Hz), a prymary methyl at δ 0.87 (t, J = 6.4 Hz), oxymethine proton at δ 3.52 (m) and an olefinic proton at δ 5.35 (dd). The C NMR spectrum of compound 2 also supported that suggestion. Based on the NMR, mass spectral data and literature [7], compound 2 was elucidated as β-sitosterol.



δ = 21 for M+ at m/z 398
δ = 22 for M+ at m/z 412
δ = 23 for M+ at m/z 426

Figure 3. Hydrolysis of compound 1a.

Acetylation of 3 afforded a tetra acetate derivative 3a. The ^{1}H NMR spectrum of 3a showed the presence of two singlet methyls, three doublet methyls, a triplet methyl, olefinic proton, oxymethine at δ 3.48 (H-3a, tt, J = 9.4, 5.8 Hz), and an anomeric proton at δ 4.59 (d, J = 7.9 Hz). The ^{13}C NMR signals were essentially the same as those of 2, except one of the hydroxyl signals, which was shifted downfield by 8.2 ppm (from δ 71.9 to 80.1). Based on the NMR and mass spectral data, compound 3 was established as 3β-O-β-Dglucopyranosylsitosterol [7].

Compounds 4, 5 and 6.

Compound 4a prepared from acetylation of compound 4 had the molecular formula C34H52O7 based on M+ at m/z 572 and showed an acetyl group at 1740 and 1240 cm^{-1} in IR spectrum. From a cursory inspection of the ^{13}C NMR spectrum, four in 9 unsaturations could be attributed to three carbonyl groups and a double bond, so that a pentacyclic system was suggested. It was assumed that the main C30 skeleton probably was triterpenoid in origin.

The ^{1}H NMR spectrum of 4a (Table 1) presented the signals of five methyl singlets at δ 0.90 (CH3-23, CH3-24), 1.1 (CH3-25), 0.73 (CH3-26), 1.26 (CH3-27), and 1.21 (CH3-29), a methyl doublet at δ 0.94 (J = 6.1 Hz), olefinic proton at δ 5.35 (H-12, t, J = 5.5 Hz), two oxymethine protons at δ 5.11 (H-2, ddd, J = 10.3, 6.1, 4.3 Hz), and δ 4.75 (H-3, d, J = 10.3 Hz), methine proton at δ 2.54 (s) as H18, and two acetyl groups at δ 2.1 (6H, s).

The ^{13}C NMR spectrum of 4a (Table 3) also indicated the presence of six methyls at δ 28.5 (CH3-23), 16.7 (CH3-24), 17.0 (CH3-25), 16.9 (CH3-26), 24.5 (CH3-27), 27.4 (CH3-29), and 16.4 (CH3-30), a carbonyl carbon at δ 183.6, two oxymethine carbons at δ 70.1 (C-2) and 80.8 (C-3), an oxycarbon at δ 73.1 (s, C19), also olefinic carbon peaks at δ 128.9 (C-12, d), 138.9 (C-13, s). That peaks were characteristic for Ursolic acid skeleton.

Table 1. ^{1}H NMR chemical shift values of compounds 4a, 5a and 6a (in CDCl3, at 40°C)

H	Compound 4a (δ, ppm)	Compound Sa (δ, ppm)	Compound 6a (δ, ppm)
2	5.11 (ddd,103, 6.0, 4.3 HZ)	5.10 (ddd, 10.4, 5.8, 4.3 HZ)	5.12 (dd, 11.6, 5.8, 4.3 Hz)
3	4.75 (d, 103 Hz)	4.73 (d, 10.4 Hz)	4.76 (d, 11.6 Hz)
12	5..35 (t, 5.5 Hz)	5..23 (t, 3.7 Hz)	5..26 (t, 3.7 Hz)
18	2.54 (s)	2.19 (d, 11.0 Hz)	2.83 (s)
23	0.90 (s)	0.90 (s)	0.93 (s)
24	0.90 (s)	0.90 (s)	0.93 (s)
25	1.10 (s)	1.06 (s)	1.07 (s)
26	0.73 (s)	0.75 (s)	0.77 (s)
27	1.26 (s)	1.13 (s)	1.13 (s)
29	1.21 (s)	0.86 (d, 8.9 Hz)	0.93 (s)
30	0.94 (d, 6.1 Hz)	0.94 (d, 6.4 Hz)	0.88 (s)
	2.10 (s, (s,CH3COO)	2.04 (s,CH3COO)	2.10 (s,CH3COO)
	2.10 (s,CH3COO)	1.97 (s,CH3COO)	2.10 (s,CH3COO)

Table 2. 1H NMR chemical shift values of compounds 7a, 8a and 9a (in CDCl3, at 40°C)

H	Compound 7a (δ, ppm)	Compound 8a (δ, ppm)	Compound 9a (δ, ppm)
3	4.50 4.55 (dd,9.4,7.4Hz)	4.50 (dd,9.4,7.3Hz)	(dd,7.3Hz)
12	5.35 (t,3.7Hz)	5.24 (t,3.7Hz)	5.27 (t,3.7Hz)
18	2.54 (s)	2.18 (d,11.3HZ)	2.83 (dd)
23	0.87 (s)	0.87 (s)	0.87 (s)
24	0.86 (s)	0.86 (s)	0.86 (s)
25	0.95 (s)	0.95 (s)	0.96 (s)
26	0.74 (s)	0.78 (s)	0.76 (s)
27	1.25 (s)	1.26 (s)	1.13 (s)
29	1.21 (s)	0.78 (d,8.6Hz)	0.97 (s)
30	0.94 (d,6.4Hz)	0.93 (d,7.6Hz)	0.91 (d,7.6Hz)
	2.04 2.04 (s,CH3COO)	2.04 (s,CH3COO)	(s,CH3COO)

Moreover, the multiplicity of the hydrogen H-18 at δ 2.54 (s) agreed with the existence of a 19α hydroxyl group. Also, the presence of hydroxyl group was corroborated by a downfield effect on CH3-29 which shifted from δ 0.78 to 1.21, as it does in methyl Urs-12-en-28-oate or Ursolic acid [8].

From the mass fragmentation data, peaks at m/z 264 and 246 (Figure 4) obtained from retro Diels Alder fragmentation [9], indicated that the double bond was located at C-12 (Figure 2). In addition the fragment at mZz 264 as constituent with the fact one hydroxyl and one carboxyl group was present on the above ring. Consequently, the other hydroxyl groups must be attached on the ring A and or B. The oxymethine (C-3) signal at o 4.75 as doublet (d, J = 10.3 H-2), indicated that the other hydroxyl group must be located at C-2 as β-hydroxyl.

Table 3. ^{13}C NMR Chemical Shif Values of Compounds 4a - 9a

	Compound					
C	4a	5a	6a	7a	8a	9a
	(δ)	(δ)	(δ)	(δ)	(δ)	(δ)
1	44.1 t	44.2 t	43.9 t	38.2 t	38.2 t	38.4 t
2	70.1 d	70.1 d	70.1 d	28.1 t	23.5 t	23.6 t
3	80.8 d	80.8 d	80.8 d	81.0 d	81.0 d	81.0 d
4	39.4 s	39.4 s	39.4 s	37.8 s	37.8 s	37.8 s
5	54.9 d	55.0 d	55.0 d	55.3 d	55.4 d	55.4 d
6	18.4 t	18.4 t	18.4 t	18.4 t	18.3 t	18.3 t
7	32.7 t	32.5 t	32.6 t	32.8 t	32.7 t	32.6 t
8	40.1 s	39.1 s	39.4 s	40.1 s	39.6 s	39.4 s
9	47.2 d	47.6 d	47.5 d	47.2 d	47.6 d	47.6 d
10	38.2 s	38.2 s	38.3 s	37.1 s	37.1 s	37.1 s
11	23.8 t	23.5 t	23.4 t	23.7 t	23.4 t	23.4 t
12	128.9 d	125.5 d	122.3 d	129.4 d	125.8 d	122.6 d
13	138.9 s	138.2 s	143.8 s	138.0 s	138.1 s	143.7 s
14	41.3 s	42.1 s	41.7 s	41.2 s	41.1 s	42.0 s
15	29.7 t	28.0 t	27.7 t	29.7 t	28.1 t	28.1 t
16	25.4 t	24.1 t	23.5 t	25.4 t	23.6 t	23.6 t
17	47.8 s	48.1 s	46.6 s	47.9 s	48.0 s	48.0 s
18	53.0 d	52.6 d	41.7 d	53.0 d	52.7 d	52.7 d
19	73.1 s	39.4 d	45.9 t	73.2 s	38.9 d	46.0 d
20	41.2 d	38.9 d	30.6 s	41.2 d	39.1 d	29.7 d
21	26.1 t	30.7 t	33.9 t	26.6 t	30.7 t	30.7 t
22	37.5 t	36.8 t	32.6 t	37.6 t	36.8 t	36.8 t
23	28.5 q	28.5 q	28.5 q	28.1 q	28.1 q	28.1 q
24	16.7 q	17.2 q	16.5 q	16.7 q	16.7 q	16.7 q
25	17.0 q	17.2 q	16.6 q	15.4 q	15.6 q	15.6 q
26	16.9 q	23.6 q	16.9 q	17.1 q	17.1 q	17.1 q
27	24.5 q	24.5 q	25.9 q	24.5 q	25.9 q	25.9 q
28	183.6 s	183.7 s	183.9 s	183.7 s	183.7 s	183.9 s
29	27.4 q	17.7 q	33.1 q	27.5 q	17.2 q	17.2 q
30	16.4 q	21.2 q	23.6 q	16.1 q	21.1 q	21.1 q
	20.9 q	20.8 q	20.8 q	21.3 q	21.3 q	21.2 q
	21.1 q	21.1 q	21.1 q	171.0 s	171.0 s	171.0 s
	170.5 s	170.5 s	170.5 s	170.8 s	170.8 s	170.8 s

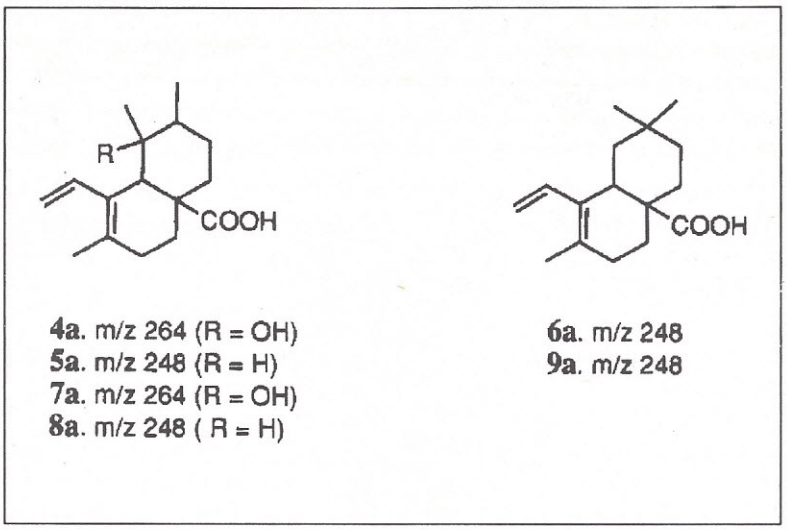


Figure 4. Mass fragmentations for compounds 4a-9a.

Comparison of the ^{13}C NMR spectrum of 4a with the a-amyrin was specially helpful, the C-18 at δ 53.0 in 4a and 58.9 in α-amyrin. This difference of up to 5.9 ppm, resulted in an upEield effect due to the C-28 carboxyl group in compound 4a. Based on these results, compound 4 was assigned as 2α,3α,19α-trihydroxy-urs-12-en-28-oic acid or 2β,19α-dihydroxyursolic acid, which also isolated from medicinal plant *Geurn Japonicurn* Thunberg [2], belonging to the Rosaceae.

The mixture of triterpenoic acids 5 and 6 was acetylated to give two diacetyl derivatives 5a and 6a. The EIMS gave molecular peak at m/z 556, corresponding to a molecular formula of C34H52O6. The presence of ester at 1740 and 1250 cm^{-1} was shown in the IR spectrum. The ^{1}H NMR spectrum data of these compounds exhibited the presence of two olefinic protons at δ 5.23 (H-12, t, J = 3.7 Hz) and 5.26 (t, H-12, J = 3.7 Hz), four oximethine protons at δ 5.10 (H-2, ddd, J = 10.4, 5.8, 4.3 Hz), 4.73 (H-3, d, J = 10.4 Hz) and 5.12 (H-2, ddd, J = 11.6, 5.8, 4.3 Hz), 4.73 (H-3, d, J = 10.4 Hz), two methine protons at δ 2.19 (H-18, d, J = 10.0 Hz), 2.83 (H-19 (dd, J = 10 Hz), two diacetyl groups at δ 2.04, 1.96 and 2.04, 1.97, one methyl doublet at δ 0.94 (J = 6.4 Hz) and many peak methyl singlets.

The ^{13}C NMR spectrum data of these compounds also observed the signals based on two oxymethine carbons at δ 70.1, 80.8 (each, C-2, C-3, d, double intensity), two double bonds at δ 125.5 (C-12, d), 138.2 (C-13, s), and 125.3 (C-12, d), 143.8 (C-13, s), two carboxyl groups at δ 183.7 (s) and 183.9 (s). Based on these data, suggested that the structures of Sa and 6a were α-amyrin and β-amyrin type triterpenoic acids having two hydroxyl groups, respectively. The methine signals (C-18) at δ 2.19 as doublet in 5a and 2.83 double of doublet in 6a, indicated that C-19 were methine and methylene, respectively, which by comparison with 4a, indicated lack of one oxycarbon in 4a. It was supported by mass fragmentation at mlz 248 and 203. Compounds 5 and 6 were then identified as 2α,3β,-dihydroxy-urs-12-en-28-oic acid (2α-monohydroxyursolic acid) and 2α,3β,-dihydroxy-olean-12-en-28-oic acid (2α-monohydroxyoleanolic acid), respectively by complete identify of the above spectral data and with those of reference [8, 10], wihich were isolated from *Geutn Japonicum. T.* [2].

Compounds 7, 8 and 9.

The Compound 7a was obtained from acetylation of 7, to give a molecular ion peak at m/z 514, corresponding to the molecular formula C32H50O5 in the EIMS data Its IR spectrum showed the absorption of ester group at 1720 and 1250 cm^{-1} . The ^{1}H NMR spectra data of 7a showed six signals of methyl singlets at δ 0.87, 0.86, 0.95, 0.74, 1.25, and 1.21, one methyl doublet at δ 0.94 (J = 6.4 Hz), an olefinic proton at δ 5.35 (H-12, t, J = 7.4 Hz), an oxymethine proton at δ 4.50 (H-3, dd, J = 9.4, 7.4 Hz), and also a methine proton CH- 18 at δ 2.54 (s). In ^{13}C

NMR it was also observed that signals, beside a carboxyl group at δ 183.7 (s). Based on these results, mass fragmentation data and reference [10], compound 7 completely was established as 3β,19α-dihydroxy-urs-12-en-28-oic acid or 19α-monohydroxyursolic acid, which has also been isolated from *Isodon Japonicus* H.

The mixture compounds 8a and 9a as monoacetate derivatives, were formulated as C32H50O4 by the EIMS (M+ at m/z 498). Its IR spectrum displayed the presence of ester at 1720 and 1250 cm^{-1} . From the ^{1}H NMR spectrum it was shown that there are two sets signals of olefinic proton H-12 at δ 5.24 (t, J = 7.0 Hz) and 5.27 (t), two oxymethine protons (H-3) at δ 4.55 (dd, J = 9.4, 7.3 Hz) and 4.50 (dd, J = 7.3 Hz). The ^{13}C NMR spectrum also displayed two sets signals of carbon-carbon double bonds at δ 125.8 (d) and 138.1 (s), and 122.6 (d) and 143.7 (s). Based on these results and reference [8], the compound 8 and 9 were assigned as 3β,-hydroxy-urs-12-cn-28-oic acid (ursolic acid) and 3β,-hydroxy-olean-12-cn28-oic acid (oleanolic acid), respectively.

CONCLUSION

An investigation of the structures of chemical constituents of methanol extract from the whole plant of *P. articulata*, have isolated known phytoceramides, N-(2'-hydroxy-acyl)-2-amino- 1 ,3,4-trihydroxy-8-octadecene, (1), two steroid compounds, identified as β-sitosterol (2), 3β-O-β-D-glucopyranosylsitosterol (3), and triterpenoic acids, elucidated as 2β,19α--dihydroxyursolic acid (4), mixture 2α-monohydroxyursolic acid (5) and 2α-monohydroxyoleanolic acid (6), 19α-monohydroxyursolic acid (7), mixture ursolic acid (8) and oleanolic acid (9) (Figure 1). All of these compounds have been isolated previously from the other medicinal plants, *Urtioca dioca* and cushion plant of *Thylacospermum caespitosum (1), Prunella vulgaris* L. var. lilacina (2 and 3), *Geum Japonicum* Thunberg (4, 5 and 6) and *Isodon Japonicus* H. (7, 8 and9).

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