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The Constituents of *Taraxacum hallaisanensis* Roots

Deuk Suk Yang, Wan Kyunn Whang and Il Hyuk Kim

College of Pharmacy, Chung-Ang University, Seoul 156-756, Korea

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Three sesquiterpene lactone compounds, two novel(1β,3β-dihydroxy-6β,11β,4α,5α,7αH-eudesm-12,6-olide-1-O-β-D-glucopyranoside, 1β,3β-dihydroxy-6β,4β,5α,7αH-eudesm-11 en-12,6-olide-1-O-β-D-glucopyranoside) and 1β,3β-dihydroxy-6β,11β,4α,5α, 7αH-eudesm-12,6-olide were isolated from the aqueous fraction of MeOH extract of the roots from *Taraxacum hallaisanensis* (Compositae) employing Amberlite XAD-2, ODS-gel, silica gel and Sephadex LH-20 column chromatographics. Another known compound, (-)-epicatechin, was isolated from the aqueous fraction of the MeOH extract. The total MeOH extract also contained phytosterol and a mixture of β-amyrin acetate, α-amyrin acetate and lupeol acetate. Structures of isolated compounds were elucidated by spectroscopic parameters of IR, Mass, ^{13}C-NMR, ^{1}H-NMR, ^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY and HMBC.

Key words : *Taraxacum hallaisanensis*, Compositae, 1β, 3β-dihydroxy-6β, 11β, 4α, 5α, 7αH-eudesm-12, 6-olide-1-O-β-D-glucopyranoside, 1β, 3β-dihydroxy-6β, 4α, 5α, 7αH-eudesm-11en-12,6-olide-1-O-β-D-glucopyranoside, 1β, 3β-dihydroxy-6β, 11β, 4α, 5α, 7αH-eudesm-12, 6-olide, (-)-epicatechin

INTRODUCTION

Taraxacum hallaisanensis (Compositae) is an indigenous plant to Korea, and roots of this species have been used for the treatment of inflammation, tonsilitis, hepatitis, pharyngolaryngitis, respiratory tract infection, choleresis, eczema and uresis in Korean folk medicine (Kim, 1992; Moon kyo Boo, 1965; Yook, 1992; Kim, 1974; Huh, 1986). Recently, intensive studies on the constituents of other Taraxacum plants roots (*Taraxacum species*) have revealed the occurrences of triterpene and sesquiterpene (Booth, 1964; Rutherford *et al.*, 1972; Hansel *et al.*, 1980; Rauwald *et al.*, 1985; Ageta *et al.*, 1981).

We previously reported the isolation and identification of three phenolic compounds from aerial parts of *Taraxacum hallaisanensis* (Whang *et al.*, 1994). As a part of our continuing study to identify bioactive compound from Korean medicinal plants, we have isolated two novel sesquiterpene lactone glucosides, along with several known compounds.

Two novel compounds, Compound II and Compound III, were designated 1β, 3β-dihydroxy-6β, 11β, 4α, 5α, 7αH-eudesm-12, 6-olide-1-O-β-D-glucopyranoside and 1β, 3β-dihydroxy-6β, 4α, 5α, 7αH-eudesm-11en-12,6-olide-1-O-β-D-glucopyranoside through their spectroscopic evidences and named hallaisanoside A, B respectively. The other known compound was isolated and identified as 1β, 3β-dihydroxy-6β, 11β, 4α, 5α, 7αH-eudesm-12, 6-olide (tetrahydroridentin B).

Correspondence to: Il Hyuk Kim, College of Pharmacy, Chung-Ang University, Seoul 156-756, Korea

MATERIALS AND METHODS

Instruments

Melting points were determined on Electrothermal IA 8100 apparatus. IR spectra was obtained with Shimadzu IR-435. Optical rotations were measured with Jasco DIP-370. ^{1}H-NMR and ^{13}C-NMR spectra were measured with a Bruker AM-200, AMX-500 at 200 and 500MHz with tetramethylsilane as an internal standard.

EI-MS was taken on a GC-MS/MS-DS, TSQ 700 mass spectrometer. FAB-MS was taken VG70-VSEQ spectrometer on condition with ionized by 35 KV Cs+ ion beam and glycerol matrix. GC was carried out to identify sugars with a Shimadzu GC-14A spectrophotometer

Plant and Material

Roots of *Taraxacum hallaisanensis* were collected in May, 1992, at Mt. Halla located Che-ju Island of Korea.

After depositing the voucher specimen at the Department of Pharmacal Botany, College of Pharmacy,

Chung-Ang University, we used air-dried and crushed roots for experiment.

Extraction and isolation

Dried material (2.5 kg) was extracted with hot MeOH. The MeOH extract was suspended in hot H_2O and partitioned succesively with $CHCl_3$ and the $CHCl_3$ layer (30 g) was subjected to silica gel column chromatography with $CHCl_3$ to give five fractions designated as $cf_1 \sim cf_5$.

Further silica gel column chromatography of cf_2 with $CHCl_3$-MeOH (1:1) afforded Compound VI (300 mg).

The cf_4 was chromatographed on silica gel column chromatography with $CHCl_3$-MeOH (9:1) and afforded Compound V (10 mg).

The H_2O soluble fraction (25 g) was applied to column paked with Amberlite XAD-2 resin. This column was eluted using mixtures of H_2O:MeOH with increasing methanol portion (0, 20, 40, 60, 80, 100%) to give six fractions. Among them, 40% MeOH fraction (5 g) was chromatographed on ODS gel with 30% MeOH to give four subfractions designated as 40-1~40-4.

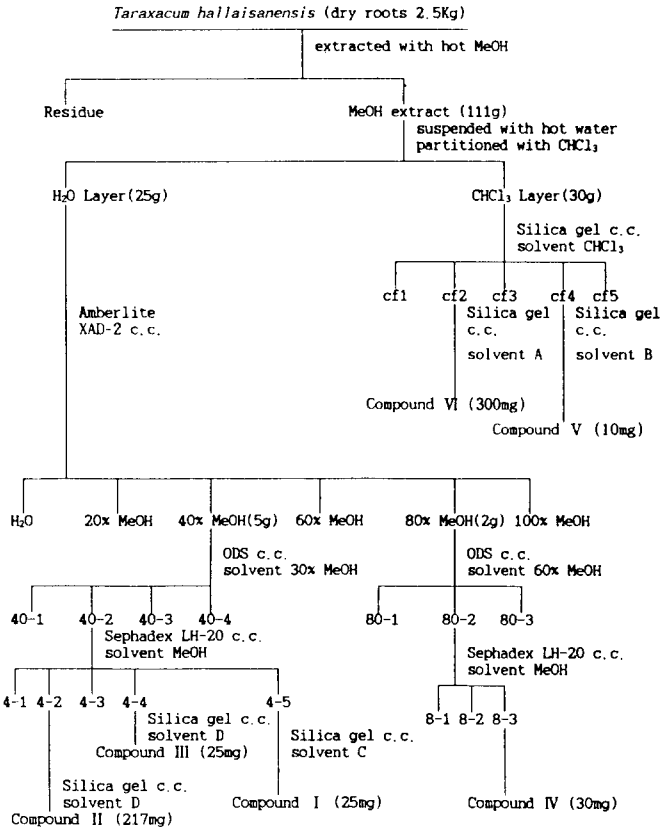
Further Sephadex LH-20 column chromatography of 40-2 with MeOH to give five fractions designated as 4-1~4-5 fraction and silica gel chromatography of 4-2 and 4-4 with $CHCl_3$-MeOH-H_2O (80:25:2.5) were afforded Compound II (217 mg) and Compound III(25 mg), respectively. And also 4-5 was subjected to silica gel column chromatography with $CHCl_3$-MeOH-H_2O(70:30:4) to yield Compound I (25 mg).

The 80% MeOH fraction (2g) was chromatographed on ODS gel with 60% MeOH to give three fractions designated as (80-1~80-3). Further Sephadex LH-20 chromatography of 80-2 with MeOH was given three fractions and among them 8-3 was afforded Compound IV (30 mg).

Compound I: m.p.: 141-142°C; Anal. Calcd. for $C_{15}H_{24}O_4$: C 67.12%, H 9.02% Found : C 67.20%, H 9.04%; IR ν_{max}^{KBr} cm^{-1}: 3326 (OH), 1775 (γ-lactone); EI-MS (m/z) : 268 $[M^+]$, 250 $[M-H_2O]^+$, 240 $[M-CO]^+$; ^{1}H-NMR : pyridine-d_5 δ ppm: see Table I; ^{13}C-NMR : pyridine-d_5 δ ppm : see Table II.

Acetylation of Compound I: Compound I (10 mg) in pyridine (2 ml) and acetic anhydride (2 ml) was allowed to stand overnight at room temperature. The mixture of reaction was diluted with ice water and then extracted with ether. The organic layer was washed with H_2O and concentrated to give compound I acetate.

Compound I Acetate: m.p.: 185°C; IR ν_{max}^{KBr} cm^{-1} : 1779 (γ-lactone), 1740, 1237 (-OAc); EI-MS(m/z) : 352 $[M^+]$, 292 $[M-HOAc]^+$, 250 $[M-HOAc-Ketone]^+$, 232 $[M-2HOAc]^+$, 217 $[M-2HOAc-CH_3]^+$, ^{1}H-NMR : pyridine-d_5 ppm : see Table I; ^{13}C-NMR : pyridine-d_5 ppm : see Table II.



Scheme 1. Extraction and Isolation of Constituents of the *Taraxacum hallaisanensis* Roots (solvent A, $CHCl_3$:MeOH=1:1; solvent C, $CHCl_3$:MeOH:H_2O=70:30:4; solvent B, $CHCl_3$:MeOH=9:1; solvent D, $CHCl_3$:MeOH:H_2O=80:25:2.5)

Compound II: m.p.: 192°C; Anal. Calcd. for $C_{21}H_{34}O_9$: C 58.57%, H 7.96%: Found : C 58.58%, H 7.97%; $[\alpha]_D$: -17.0 (c=0.30 in MeOH); IR ν_{max}^{KBr} cm^{-1} : 3404 (OH), 1759 (γ-lactone), 1077, 1025 (glycosidic C-O); FAB-MS (positive) (m/z) : 453 $[M+Na]^+$, 431 $[M+H]^+$, 269 $[(M+H)\text{-glc}]^+$, 251 $[M-(H+glc+H_2O)]^+$; ^{1}H-NMR : pyridine-d_5 δ ppm : see Table I; ^{13}C-NMR : pyridine-d_5 δ ppm : see Table II.

Enzymatic hydrolysis of Compound II: Compound II (20 mg) was suspended in water with DMSO (2 ml) and treated with β-glucosidase (20 mg, Sigma Chemical Company E.C 3.21.21) for 24hr. at 38° with stirring. The solution was heated at 5 min. and extracted 3 times with $CHCl_3$ to yield aglycone (10 mg) and gylcone part.

Aglycone of Compound II: IR ν_{max}^{KBr} cm^{-1} : 3326 (OH), 1775 (γ-lactone); EI-MS (m/z) : 268 $[M^+]$, 250 $[M-H_2O]^+$, 240 $[M-CO]_+$; ^{1}H-NMR : pyridine-d_5 δ ppm: 1.19 (3H, d, J=10.1 Hz, 13-H), 1.25 (3H, s, 14-H), 1.30 (3H, d, J=7.4 Hz, 15-H): Table I; ^{13}C-NMR : pyridine-d_5 δ ppm: see Table II.

Sugar part of Compound II: GC : TMS standard glu-

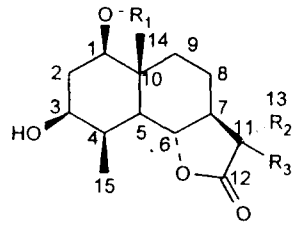
Table I. ^{1}H-NMR Spectra of Compound I, I-Ac, II III, II-aglycone (pyridine-d_5, 200, 500 MHz)

Proton No.	Comp. I	Comp. I-Ac	Comp. II-aglycon	Comp. II	Comp. III
H-1α	ddd 3.62	dd 4.82	ddd 3.64	ddd 3.64	ddd 3.70
H-2α	ddd 1.45	m 1.45	ddd 1.45	ddd 2.13	2α 2.32
H-2β					2β 2.22
H-3α					3α 4.35
H-3β	ddd 3.61	ddd 5.05	ddd 3.65	ddd 3.94	ddd 4.35
H-4	ddq 2.27	ddq 2.45	ddq 2.45	ddq 2.49	ddq 3.15
H-5α	dd 1.43	dd 1.45	dd 1.45	dd 1.45	dα 1.45
H-6β	dd 4.20	dd 4.17	dd 4.20	dd 4.06	dd 4.30
H-7α	dd(b4) 1.51	dd(br) 1.60	dd 1.55	dd(br) 1.41	dd 2.85
H-8α	m 1.64	m 1.50	m 1.65	m 1.49	m 1.80
H-8β	dddd 1.53	dddd 1.45	dddd 1.50	dddd 1.43	dddd 1.65
H-9α	ddd 1.28	dd 1.88	ddd 1.25	ddd 2.07	ddd 2.75
H-9β	d 1.32	d 1.65	d 1.35	d 2.05	d 2.65
H-11	dq 2.65	dq 2.35	dq 2.75	dq 1.13	
H-13	d 1.30	d 1.28	d 1.15	d 1.06	α 6.31
					β 5.81
H-14	s 1.19	s 1.05	s 1.20	s 1.12	s 1.25
H-15	d 1.25	d 1.07	d 1.25	d 0.97	d 1.09
H-1'				4.82	4.90
H-2'				4.04	3.95
H-3'				3.83	3.85
H-4'				4.20	4.22
H-5'				4.35	4.35
H-6'				4.55	4.45
				4.65	4.55
OAc		s 2.06			
OAc		s 2.02			

Compound I, Compound II: J(Hz):1α,2α=4,5; 1α,2β=11,5; 2α,3α=5,5 2β,3α=11,3α,4α=5,5; 4α,15=7; 4α,5α=4,5; 5α,6β=11; 6β,7α=10; 7α,8β=12; 7α,11β=11; 8α,8β=12; 8β,9α=12; 8β,9β=3; 11,13=11

Table II. ^{13}C-NMR Spectra of Compound I, II, III and II-aglycone (pyridine-d_5, 50, 125 MHz)

Carbon No.	Compound I	Compound I-Ac	Compound II-aglycone	Compound II	Compound III
C-1	77.3	77.9	77.2	84.2	84.9
2	40.4	31.5	40.4	40.2	40.5
3	70.4	72.2	70.4	70.2	71.2
4	41.9	39.3	41.8	41.9	34.8
5	53.7	53.1	53.7	53.6	51.5
6	79.9	78.5	79.9	79.7	69.4
7	48.0	47.4	48.0	48.2	50.9
8	23.4	23.0	23.4	23.2	27.7
9	36.6	28.4	36.7	35.4	32.5
10	42.1	41.5	42.1	41.2	40.4
11	41.8	41.0	41.8	41.8	145.0
12	179.6	179.0	179.6	179.5	168.6
13	12.9	12.7	12.8	12.8	124.6
14	15.7	15.6	15.7	16.1	16.6
15	9.6	9.3	9.6	9.5	9.6
C'-1				102.6	102.6
2				75.2	75.2
3				78.3	78.3
4				72.0	71.9
5				78.7	78.8
6				63.2	63.2
-OCOCH$_3$		169.7			
-OCOCH$_3$		170.1			
-OCOCH$_3$		20.4			
-OCOCH$_3$		20.8			



	R_1	R_2	R_3
Compound I	H	CH_3	H
Compound II	Glc	CH_3	H
Compound III	Glc	CH_2	

Fig. 1. structures of Compound I, II and III

cose (t_R 9.45, 11.90): Sample (t_R 9.28, 12.13); GC condition : Column : OV-1 capillary column (25 m): Column temperature : 220°C (5°C/2 min): Carrier gas : H_2 gas (12 ml /min): Detector : FID

Compound III: m.p : 184°C : Anal. Calcd. for $C_{21}H_{32}O_9$: C 58.87%, H 7.53%, Found : C 58.88%, H 7.52%; IR ν_{max}^{KBr} cm^{-1} : 3409 (OH), 1761 (γ-lactone), 1072, 1040 (glycosidic C-O), 1626 (C=C); FAB-MS (negative) (m/z) : 427 $[M-H]^-$; HR-MS (m/z) : 428.9873 $[M^+]$; ^{1}H-NMR : pyridine-d_5 δ ppm: 4.90 (1H, d, *J*=7.7 Hz, anomeric H): see Table I; ^{13}C-NMR : pyridine-d_5 δ ppm : see Table II

Compound IV: m.p.: 246-248°C; Anal. Calcd. for $C_{15}H_{14}O_6$: C 62.05%, H 4.87%, Found : C 61.88%, H 4.76%; $[\alpha]_D$: -54.0 (c=0.5 in EtOH); IR ν_{max}^{KBr} cm^{-1} : 3456 (OH), 1625, 1521, 1469 (aromatic C=C); EI-MS (m/z) : 290 $[M^+]$, 272, 152, 139; ^{1}H-NMR : DMSO-d_6 δ ppm: 2.47 (1H, dd, *J*=6,8 Hz, H-4), 3.99 (1H, *m*, H-3), 4.64 (1H, *brs*, H-2,), 5.75 (1H, d, *J*=2.2 Hz, H-6), 5.87 (1H, d, *J*=2.2 Hz, H-8), 6.65 (2H, *brs*, H-5',6'), 6.93 (1H, *brs*, H-2'); ^{13}C-NMR : DMSO-d_6 δ ppm :

Compound V: IR ν_{max}^{KBr} cm^{-1} : 3420 (OH), 1652 (C=C), 1472 (CH_2); GC-MS (m/z) : 414 $[M^+]$, 412 $[M^+]$, 400 $[M^+]$; Gas chromatography : Column : OV-17 (2.6 mm × 1.1 m), Column temp.: 250°C-290°C, Injection temp.: 270°C, Carrier gas: N_2, Flow rate : 60 ml/min, Detector : FID; ^{1}H-NMR : pyridine-d_5 δ ppm: 0.67 (3H, s, CH_3), 0.85-0.89 (9H, *m*, CH_3), 0.97 (3H, s, CH_3), 1.06 (3H, s, CH_3), 3.85 (1H, *m*, H-3), 5.42 (1H, d, *J*=4.1 Hz, H-6).

Compound VI: IR ν_{max}^{KBr} cm^{-1} : 1734, 1246 ($OCOCH_3$); ^{1}H-NMR : pyridine-d_5 ppm: 0.82-1.73 (*m*, CH_3), 2.05 (3H, *s*, OAc), 4.73 (1H, *m*, H-3), 5.20 (1H, *m*, H-12); GC-Mass (m/z) : 468 $[M^+]$, 408 $[M-OCOCH_3]^+$; GC-MS condition : Column : OV-1 capillary column (25 m), Column Temp.: 300°C, Carrier gas : He gas (1 ml/min.), Detector : FID

RESULTS AND DISCUSSION

The water layer of the methanol extract yielded the following known sesquiterpene lactone Compound I (tetrahydroridentin B), in addition to the new sesquiterpene lactone glycosides, Compound II, Compound III and compound IV is epicatechin.

Another part of the methanol extract, the chloroform fraction yielded the mixture of pentacyclic triterpene acetate and phytosterol

Compound I was obtained as an amorphous powder, I.R. spectrum of Compound I gave 3326 (OH) and 1775 (γ-lactone) cm^{-1}. In the EI-MS spectrum of Compound I, molecular and fragment ion peak appeared at m/z 268 $[M^+]$, 250 $[M-H_2O]^+$ and 240 $[M-CO]^+$.

In the ^{1}H-NMR spectrum of Compound I (Table I), the signals of three angular methyl group showed two doublets at 1.25, 1.30 (H-15, H-13) and one singlet 1.19 (H-14).

The germinal signal to the free hydroxyl group which was appeared lower field δ 3.62 (H-1), δ 3.61 (H-3) and also lactonic methine proton displayed δ 4.17 (H-6, *J*=10.0, 10.0 Hz *dd*) (Sanz *et al.*, 1991; Fischer *et al.*, 1979).

The signals of H-1, 3, 6 were not only confirmed the proposed structure but also led to the assignment of all ^{1}H-NMR signals of tetrahydroridentin B (Hansel *et al.*, 1980).

After acetylation of Compound I, the ^{1}H-NMR spectrum indicated two angular methyl doublets at δ 1.07, 1.28 (H-15, H-13) and one angular methyl singlet 1.05 (H-14) and H-1, H-3 methine protons were shifted downfield from δ 4.58 to 4.82 (*J*=4.8, 11.0 Hz, *dd*) and from δ 4.82 to 5.05 (5.5, 5.5, 11.5 Hz, *ddd*), respectively, and suggested that C-1 and C-3 were conjugated at hydroxyl group and also coupling pattern of proton signal (δ 4.82 *dd*) assigned axial proton (H-1α) and more downfield (δ 5.05, *ddd*) assigned axial proton (H-3α).

From those results, assigned 1β, 3β stereochemistry between H-1 and H-3 and additionally coupling constant of H-3 axial proton (5.5, 5.5, 11.5 Hz) suggesting that H-4 proton connected to equatorial and 15-CH_3 proton conjugated to axial position.

Between the ^{13}C-NMR and DEPT 135° spectrum, these signals showed three angular methyls (δ 9.6, 12.9, 15.7), three methylenes (δ 40.4, 23.4, 36.6), seven methines (δ 77.3, 70.4, 41.9, 53.7, 79.9, 48.0, 41.8) and two tetrasubstituted carbons (δ 179.6, 42.1).

Lactonic carbonyl carbon showed C-12 (δ 179.6) and lactone ethereal oxygen showed C-6 (δ 79.9) and also two oxygenated carbon signal located at C-1 (δ 77.3), C-3 (δ 70.4).

From the result of the ^{13}C-NMR data, Compound I proposed the decaline system of eudesman sesquiterpene lactone (Marco *et al.*, 1987).

The identification of Compound I was confirmed by ^{13}C-NMR, ^{1}H-NMR, ^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY spectrum and EI-MS spectrum and by comparison of the

reported data (Holub, 1984; Yamakawa *et al.*, 1976; Geissman *et al.*, 1969; Sanz *et al.*, 1990; Irwin *et al.*, 1973; Mahmound *et al.*, 1981; Kouno *et al.*, 1990).

In the all results, Compound I was identified as 1β, 3β-dihydroxy-6β, 11β, 4α, 5α, 7αH-eudesm-12, 6-olide(tetrahydroridentin B).

Compound II was obtained as an amorphous powder, I.R. spectrum of Compound II gave 3404 (OH), 1759 (γ-lactone) and 1077, 1025 (glycosidic C-O) cm^{-1}. In the FAB-MS (positive) spectrum of Compound II, molecular and fragment ion peak appeared at m/z 453 $[M+H+Na]^+$, 431 $[M+H]^+$ and 269 [M+H-Glc]$^+$.

The ^{1}H-NMR spectrum (pyridine-d_5, Table I) showed three angular methyls at at 0.97, 1.12, 1.06 (H-15, 14, 13), three methylenes at 2.13, 1.49, 1.43, 2.07, 2.05 (H-2, 8α, 8β, 9α, 9β), seven methines at 3.64, 3.94, , 2.49, 1.45, 4.06, 1.41, 1.13(H-1, 3, 4, 5, 6, 7 and 11) and anomeric proton signal at δ 4.82 (1H, d).

By the ^{13}C-NMR, ^{1}DEPT 135°, ^{1}H-^{1}H COSY and ^{13}C-^{1}H COSY spectrum, these signals identified three angular methyls (δ 9.5, 12.8, 16.1), three methylenes (δ 40.2, 23.2, 35.4), seven methines (δ 84.2, 70.2, 41.9, 53.6, 79.7, 48.2, 41.8), two tetrasubstituted carbons (δ 179.5, 41.2), anomeric carbon (δ 102.6) and also five glucosyl carbons (δ 75.2, 78.3, 72.0, 78.7, 63.2), and especially the approach of HMBC and NOE spectrum were confirmed that 1 mole of glucose was conjugated C-1 of aglycone.

On the enzymatic hydrolysis with glucosidase, aglycone of Compound II was identifird tetrahydroridentin B. by comparison of reported data, spectral data and Compound I (Irie *et al.*, 1990; Fraga *et al.*, 1993; Ushima *et al.*, 1993; Goren *et al.*, 1993).

In the results, Compound II was 1β, 3β-dihydroxy-6β, 11β, 4α, 5α, 7αH-eudesm-12, 6-olide-1-O-β-D-glucopyranoside.

Compound III was obtained as a amorphous powder. I.R. spectrum of Compound III gave 3409 (OH), 1761 (γ-lactone) and 1040 (glycosidic C-O) cm^{-1}. In the FAB-MS (negative) spectrum of Compound III molecular ion and fragment ion peak appeared at m/z 427 $[M-H]^-$ and 265 $[M-(H+glc)]^-$ and also molecular ion peak of HR-MS appeared at m/z 428.9873 suggesting that the formular of Compound III is $C_{21}H_{32}O_9$.

The ^{1}H-NMR spectrum (pyridine-d_5, Table I) showed two angular methyls at δ 1.09, 1.25 (H-15, 14), four methylenes at δ 2.32, 2.22, 1.80, 1.65, 2.75, 2.65, 6.31, 5.81 (H-2α, 2β, 8α, 8β, 9α, 9β, 13α, 13β), six methines at δ 3.70, 4.35, 3.15, 1.45, 4.30, 2.85 (H-1, 3, 4, 5, 6 and 7) and anomeric proton signal at δ 4.90 (1H, d).

By the ^{13}C-NMR, DEPT 135°, ^{1}H-^{1}H COSY and ^{13}C-^{1}H COSY spectrum, these signals identified two angular methyls (δ 9.6, 16.6), four methylenes (δ 40.5, 27.7, 32.5, 124.6), six methines (δ 84.9, 71.2, 34.8, 51.5, 69.4, 50.9), three tetrasubstituted carbons (δ 168.6, 145.0, 40.4), anomeric carbon (102.6) and also five glucosyl carbons (δ 75.2, 78.3, 71.9, 78.8, 63.2), and especially the approach of HMBC and NOE spectrum were confirmed that the structure showed typical singlet signal at δ 6.31, 5.81 ppm due to the exocyclic methylene group and also anomeric proton signal of glucose at 4.90 ppm (*J*=7.4Hz, *d*) must be conjugated C-1 of aglycone.

On the enzymatic hydrolysis with glucosidase, Compound III gave glucose and aglycone, and identified by comparison of reported data and spectral data (Arias *et al.*, 1987; Harapanhall *et al.*, 1988; Lee *et al.*, 1970; Brauno *et al.*, 1988; Miyase *et al.*, 1984; Jimenez *et al.*, 1993).

In the results, Compound III was 1β, 3β-dihydroxy-6β, 4α, 5α, 7αH-eudesm-11en-12,6-olide-1-O-β-D-glucopyranoside.

Compound IV was obtained as a yellow needle crystal. I.R. spectrum of Compound IV gave 3456(OH), 1625, 1525, 1470 (C=C)cm^{-1}. In the EI-MS spectrum of Compound IV, molecular and fragment ion peak appeared at m/z 290, 272, 152 (RDA fragmant with B ring) and 139 (RDA fragmant with A ring). All these data were in aggrement with those for structure of catechin derivatives.

^{1}H-NMR spectrum (DMSO-d_6) showed typical proton signals of flavanol at 4.64 (1H, *s*, H-2), 3.99 (1H, *m*, H-3) and 2.47 (2H, *dd*, *J*=6.8 Hz, H-4) to the exocyclic methylene group and additionally at 6.65 (2H, *brs*, H-5',6'), 6.93 (1H, *s*, H-2'), 5.75 (1H, *d*, *J*=2.2 Hz, H-6) and 5.87 (1H, *d*, *J*=2.2 Hz, H-8).

^{13}C-NMR spectrum (DMSO-d_6) showed exocyclic methylen group signals at δ 28.4 (C-4), 65.1 (C-3) and 78.3 (C-2). Also the C-3' and C-4' of the B ring appeared at δ 144.7 and δ 144.6 respectively indicating substitution.

These data suggested that Comound IV was epicatechin and identified by comparison of reported data, spectral data and authentic sample (Porter *et al.*, 1982; Young *et al.*, 1987).

Compound V was detected by Liebermann-Burchard positive reaction and obtained as a colourless needle and also I.R. spectrum of Compound V gave 3450 (OH) and 2950, 1470, 1380 (CH_3), 1050 (C-O) cm^{-1}.

The data of ^{1}H-NMR and ^{13}C-NMR spectrum indicated the presence of mixture of phytosterol.

GC-EI MS showed m/z 414, 412, 400 which are molecular ion peak.

These data suggested that Comound V were the mixture of β-sitosterol, stigmasterol and campesterol and identifyed by comparison of reported data, spectral data and authentic sample (Akihira *et al.*, 1986; Garg *et al.*, 1984; Nes *et al.*, 1992).

Compound VI was detected by Liebermann-Burchard positive reaction and obtained as a colourless needle and also I.R. spectrum of Compound V gave 3448 (OH) and 2944, 1457, 1367 (CH_3), 1734, 1246 ($OCOCH_3$), 1027 (C-O)cm^{-1}.

The data of ^{1}H-NMR and ^{13}C-NMR spectrum indicated the presence of mixture of pentacyclic triterpene.

GC-MS spectrum showed 3 peaks and also all of Mass spectrum of Compound VI showed molecular ion peak at m/z 468 suggesting that this compound was a mixture of triterpenoid. Two of them clearly showed m/z 218 (base peak) and 203 fragment ion peak supposed to be a pentacyclic triterpenoid having 12-double bond and an amyrin group and last peak of this compound was lupeol acetate.

In the results, Compound VI were a mixture of pentacyclic triterpene , α-amyrine acetate, β-amyrin acetate and lupeol acetate, and identified by comparison of reported data and spectral data (Bass *et al.*, 1992; Radosevich *et al.*, 1985; Buldzikiewicz *et al.*, 1963).

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