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Pentacyclic Triterpenoids from *Ilex macropoda*

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Six compounds were isolated from the twigs of *Ilex macropoda*. Their structures were elucidated as betulinic acid, lupeol, betulone, betulin, erythrodiol and 11-oxo-erythrodiol by physicochemical and spectroscopic analysis. Among them, lupeol, betulone, erythrodiol and 11-oxo-erythrodiol were isolated for the first time from this plant.

Key words: *Ilex macropoda*, Aquifoliaceae, Lupeol, Betulone, Betulin, Erythrodiol and 11-oxo-erythrodiol, Betulinic acid

INTRODUCTION

Ilex species are genus of the family Aquifoliaceae consisting of more than 300 species. *Ilex macropoda* Miq. is a tree distributed in Korea, Japan and China (Kim, 1995). Earlier investigation on the chemical constituents of *Ilex* species dealt with saponins (Kakuno *et al.*, 1991; Taketa *et al.*, 2000; Ouyang *et al.*, 2001) and phenolic compounds (Filip *et al.*, 2001). Fifteen compounds (three hemiterpenes, betulin, acetyl ursolic acid, ilexosides XVII and XVII from the bark, rotundionic acid, urosolic acid, ilexosides II and XXX ziyu-glycoside I and rutin from fresh leaves, and 3,5-dicaffeoylquinic acid and 3,4-dicaffeoylquinic acid from the wood were reported from *I. macropoda* (Fuchino *et al.*, 1997). A literature survey revealed that no pharmacological studies had been carried out on this plant. Therefore, we were interested in the chemical constituents of this plant for pharmacological study.

The chromatographic separation of the methanol extract from the twigs of *I. macropoda* led to the isolation of six pentacyclic triterpenoids, lupeol, betulone, betulin, erythrodiol, 11-oxo-erytrhodiol and betulinic acid. Among them, lupeol, betulone, erythrodiol and 11-oxo-erythrodiol were isolated for the first time from this plant.

This paper describes the isolation and structural determination of these compounds.

MATERIALS AND METHODS

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General experimental procedures

^{1}H- and ^{13}C-NMR spectra were determined on a JEOL JMN-EX 400 spectrometer in $CDCl_3$ or CD_3OD. The EI/MS(70 eV) were determined on a VG-VSEQ mass spectrometer (VG Analytical, UK). The IR spectra were obtained on a JASCO FT/IR 410 spectrometer. TLC was carried out on Merck precoated silica gel F_{254} plates and silica gel for column chromatography was Kiesel gel 60 (230-400 mesh, Merck). And Sephadex LH-20 was used for column chromatography (Pharmacia, 25-100 μm). Column for LPLC was Lobar A (Merck Lichroprep Si 60, 240-10 mm). All other chemicals and solvents were analyticlal grade and used without further purification

Plant materials

The aerial parts of *I. macropoda* were collected in October 2000 at Moak mountain, Chonbuk, Korea. A voucher specimen is deposited at the herbarium of college of pharmacy, Woosuk University, Korea (WSU-00-005).

Extraction and isolation

The air-dried plant materials (1.0 Kg) was extracted twice with hot MeOH under reflux. The resultant MeOH extract (210 g) was suspended in water, and then fractionated successively with equal volumes of CH_2Cl_2, ethyl acetate and *n*-BuOH, leaving residual water soluble fraction. Each fraction was evaporated in vaccuo to yield the residues of CH_2Cl_2 soluble fraction (40 g), ethyl acetate soluble fraction (12 g) and *n*-BuOH soluble fraction (65 g). The CH_2Cl_2 soluble fraction was chromatographed over silica gel column using stepwise gradient eluting with a gradient of $CHCl_3$-EtOAc (10:1→1:1) to give five subfractions, subfraction two was

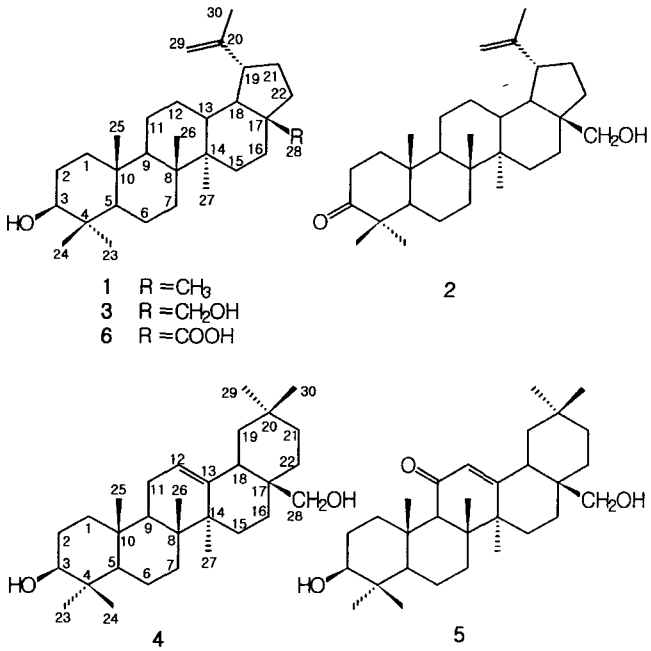

Fig. 1. The structures of compounds **1-6**

Table I. ^{13}C-NMR spectral data of six compounds 1-6 from the twigs of *Ilex macropoda*

Carbon	1[a]	2[a]	3[a]	4[a]	5[a]	6[b]
1	38.7	39.5	38.8	38.6	39.1	39.8
2	27.4	33.4	27.4	27.2	27.3	28.6
3	78.9	218.2	79.0	79.0	78.7	79.7
4	38.8	47.3	38.9	38.7	39.1	39.9
5	55.3	54.8	55.3	55.2	54.9	56.7
6	18.3	19.0	18.3	18.3	17.5	19.4
7	34.2	34.1	34.2	32.6	32.7	34.9
8	40.8	40.8	40.9	39.8	43.4	40.8
9	50.4	49.7	50.4	47.6	61.7	51.8
10	37.1	36.8	37.2	36.9	36.9	38.2
11	20.9	21.0	20.8	23.5	200.2	21.6
12	25.1	25.1	25.2	122.4	128.2	26.4
13	38.0	37.3	37.3	144.2	169.5	38.7
14	42.8	42.7	42.7	41.7	45.4	42.9
15	27.4	26.6	27.0	25.5	25.8	31.6
16	35.5	29.1	29.2	22.0	21.5	33.6
17	42.9	47.7	47.8	36.9	37.0	56.8
18	48.3	48.6	48.7	42.3	42.6	47.6
19	47.9	47.7	47.8	46.5	44.9	49.5
20	150.9	150.3	150.5	31.0	30.6	151.9
21	29.8	29.7	29.7	34.0	33.8	30.8
22	39.9	33.9	34.1	31.0	31.0	38.1
23	28.0	27.0	28.1	28.0	28.1	28.8
24	15.3	21.3	15.4	15.5	15.5	15.9
25	16.1	15.9	16.1	15.6	16.4	16.3
26	15.9	15.7	16.0	16.7	18.6	16.7
27	14.5	14.6	14.8	25.9	23.4	15.2
28	18.0	60.4	60.5	69.7	69.6	181.8
29	109.3	109.7	109.7	33.2	32.9	110.2
30	19.3	19.6	19.1	23.6	23.4	19.5

[a]Recorded at 100 MHz in $CDCl_3$.
[b]Recorded at 100 MHz in CD_3OD.

rechromatographed on silica gel column (*n*-hexane-EtOAc, 4:1) and purified by Sephadex LH-20 (MeOH) to yield **1** (10 mg) and **2** (11 mg). Subfraction four was rechromatographed on silica gel column with *n*-hexane-EtOAc (2:1) to give four fractions. Fourth fraction was chromatographed on silica gel ($CHCl_3$:CH_2Cl_2, 1:3) to yield **3** (15 mg) and **4** (12 mg). Subfraction five was applied over silica gel eluting with $CHCl_3$-EtOAc (3:1) to give **5** (10 mg). Ethyl acetate soluble fraction was applied over silica gel column using a solvent system of $CHCl_3$-MeOH (4:1) as an eluent to give three subfractions, subfraction one was afforded 20 mg of **6** by Lobar-A column ($CHCl_3$:EtOAc, 1:1).

Compound 1 (lupeol): EIMS (*m/z*): 426 [M$^+$], 411, 296, 218, 207, 189, 71, IR ν max (KBr) cm^{-1}: 3320 (-OH), 1650, 1515, ^{1}H-NMR (400MHz, $CDCl_3$) δ: 4.68 and 4.55 (each 1H, m, H-29), 3.18 (1H, dd, *J*=10.2, 4.0, H-3), 1.68 (3H, s, H-30), 1.02 (3H, s, H-26), 0.98 (3H, s, H-23), 0.96 (3H, s, H-27), 0.85, 0.80 and 0.77 (each 3H, s, H-25, 28, 24). ^{13}C-NMR (100MHz, $CDCl_3$) see the Table 1.

Compound 2 (betulone): EIMS (*m/z*): 440 [M$^+$], 409, 397, 286, 245, 203, 189, 147, 133, 119, 95, 67, 55, IR ν max (KBr) cm^{-1}: 3455 (-OH), 1705, 1615, ^{1}H-NMR (400MHz, $CDCl_3$) δ: 4.73 and 4.59 (each 1H, m, H-29), 3.78 and 3.32 (each 1H, d, *J*=11.1, H-28), 2.40 (2H, m, H-2), 1.67, 1.06, 0.99, 0.95, 0.93 and 0.90 (each 3H, s, H-23, 24, 25, 26, 27, 30). ^{13}C-NMR (100MHz, $CDCl_3$) see the Table 1.

Compound 3 (betulin): EIMS (*m/z*): 442 [M$^+$], 411, 234, 207, 203, 71, IR ν max (KBr) cm^{-1}: 3350 (-OH), 1630, ^{1}H-NMR (400MHz, $CDCl_3$) δ: 4.70 and 4.59 (each 1H, m, H-29), 3.77 and 3.30 (each 1H, d, *J*=11.0, H-28), 3.18 (1H, dd, *J*=10.1, 4.7, H-3), 1.67, 0.99, 0.96, 0.91, 0.81 and 0.75 (each 3H, s, H-30, 27, 26, 23, 25, 24). ^{13}C-NMR (100MHz, $CDCl_3$) see the Table 1.

Compound 4 (erythrodiol): EIMS (*m/z*): 442 [M$^+$], 425, 424, 234, 216, IR ν max (KBr) cm^{-1}: 3570, 1375, ^{1}H-NMR (400MHz, $CDCl_3$) δ: 5.22 (1H, m, H-12), 4.17 (2H, m, H-28), 3.38 (1H, dd, *J*=11.5, 4.6, H-3), 1.21, 0.99, 0.94, 0.87, 0.86, 0.80 and 0.78 (each 3H, s, H-23, 24, 25, 26, 27, 29, 30). ^{13}C-NMR (100MHz, $CDCl_3$) see the Table 1.

Compound 5 (11-oxo-erythrodiol): EIMS (*m/z*): 456 [M$^+$], 441, 248, IR ν max (KBr) cm^{-1}: 3420 (-OH), 1615, ^{1}H-NMR (400MHz, $CDCl_3$) δ: 5.57 (1H, s, H-12), 3.46 (1H, d, *J*=11.1, H-28), 3.25 (1H, m, H-3), 3.22 (1H, d, *J*=11.1, H-28), 2.77 (1H, m, H-1), 1.38 (3H, s, H-27), 1.12 (3H, s, H-25), 0.99 (3H, s, H-26), 0.90 (3H, s, H-23), 0.88 (3H, s, H-30) and 0.79 (3H, s, H-29). ^{13}C-NMR (100MHz, $CDCl_3$) see the Table 1.

Compound 6 (betulinic acid): EIMS (*m/z*): 456 [M$^+$],

438, 248, 228, 207, 189, IR ν max (KBr) cm^{-1}: 3510 (-OH), 1710, 1615, ^{1}H-NMR (400MHz, CD_3OD) δ: 4.67 and 4.56 (each 1H, d, *J*=2.0, H-29), 3.12 (1H, m, H-3), 1.68, 1.15, 0.97 0.95, 0.94 and 0.77 (each 3H, s, H-23, 24, 25, 26, 27, 30). ^{13}C-NMR (100MHz, CD_3OD) see the Table 1.

RESULTS AND DISCUSSION

In the course of phytochemical study of the MeOH extract from the twigs of *I. macropoda*, six pentacyclic triterpenoids lupeol (**1**), betulone (**2**), betuline (**3**), erythrodiol (**4**), 11-oxo-erythrodiol (**5**) and betulinic acid (**6**) were isolated from the CH_2C_2 and ethyl acetate soluble fractions.

Compounds **1-6** have similar patterns in their NMR spectra. Compound **1** was obtained as a white amorphorous powder from MeOH, and showed positive result in Liebermann-Burchard test. The EIMS of **1** showed an [M$^+$] ion at *m/z* 426. The IR spectrum exhibited a hydroxy (3320 cm^{-1}) absorption band. The ^{1}H-NMR spectrum of **1** showed signals for an isopropylene function (δ 4.68 and 4.55 each 1H, m, and 1.68, 3H, s) and six methyl groups (δ 1.02, 0.98, 0.96, 0.85, 0.80 and 0.77, each 3H, s). The double of doublets (*J*=10.2, 4.0 Hz) of proton with oxygenated carbon at δ 3.18 showed a 3β-hydroxyl functionality. The ^{13}C-NMR spectrum of **1** exhibited the presence of 30 carbon signals and also showed two olefinic signals at δ 150.9 and 109.3, and one oxygenated carbon signal of C-3 at δ 78.9. From these results, compound **1** indicated to be a 3β-hydroxy lupane type triterpene. The structure of **1** was determined to be 3β-hydroxylup-20(29)-ene (lupeol) on the basis of the above evidences, together with a comparison of the above data with those published in the literature (Ito *et al.*, 1978; Ahmad *et al.*, 1994).

Compound **2** was obtained as a white amorphorous powder from MeOH, and showed positive result in Liebermann-Burchard test. The EIMS of **2** showed an [M$^+$] ion at *m/z* 440. The IR spectrum exhibited a hydroxy (3455 cm^{-1}) absorption band. The ^{1}H-NMR spectrum of **2** showed signals for an isopropylene function (δ 4.73 and 4.59 each 1H, m, and 1.67, 3H, s), a set of geminal protons at δ 3.78 and 3.32 (each 1H, d, *J*=11.1 Hz, H-28), and five methyl groups (δ 1.06, 0.99, 0.95, 0.93 and 0.90, each 3H, s). The ^{13}C-NMR spectrum of **2** was similar to that of **1**, suggesting it has the similar carbon skeleton. The main difference was C-3 and C-28 chemical shift. The ^{13}C-NMR spectrum of **2** exhibited the presence of 30 carbon signals and also showed a carbonyl signal, two olefinic signals at δ 150.3 and 109.7, and a oxygenated carbon signal of C-28 at δ 60.4. From these results, compound **2** indicated to be a 3-oxo lupane type triterpene. Besides the above evidences, by the direct comparison of its spectral data with those of the reported literature, the structure of **2** was determined to be lup-20(29)-ene-28-ol-3-one (betulone), which has been isolated from *Betula lenta* (Cole *et al.*, 1991).

Compound **3** was obtained as a white amorphorous powder from MeOH, and showed positive result in Liebermann-Burchard test. The EIMS of **3** showed an [M$^+$] ion at *m/z* 442. The IR spectrum exhibited a hydroxy (3350 cm^{-1}) absorption band. NMR spectra of **3** was very similar to that of **2**, suggesting the same skeleton. The main differences were the presence δ 3.18 ppm resonance in ^{1}H-NMR spectrum and the presence of δ 79.0 ppm resonance instead of δ 218.2 ppm of **2** in ^{13}C-NMR spectrum which can be assigned to carbinol carbon (C-3). The proton with oxygenated carbon at δ 3.18 (dd, *J*=10.1, 4.7 Hz) showed a 3β-hydroxyl functionality. On the basis of the above evidences, the structure of **3** was determined to be 3β,28-dihydroxylup-20(29)-ene (betulin), together with a comparison of the above data with those published in the literature (Patra *et al.*, 1988; Siddiqui *et al.*, 1988).

Compound **4** was obtained as a white amorphorous powder from MeOH, and showed positive result in Liebermann-Burchard test. The EIMS of **4** showed an [M$^+$] ion at *m/z* 442. The IR spectrum exhibited a hydroxy (3570 cm^{-1}) absorption band. The ^{1}H-NMR spectrum of **4** revealed the presence of single olefinic proton (δ 5.22, m, H-12), seven tertiary methyl groups (δ 4.68 and 4.55, each 1H, m, and 1.68, 3H, s) and six methyl groups (δ 1.02, 0.98, 0.96, 0.85, 0.80 and 0.77, each 3H, s). The double doublets (*J*=11.5, 4.6 Hz) of proton with oxygenated carbon at δ 3.18 showed a 3β-hydroxyl functionality. The ^{13}C-NMR spectrum of **4** exhibited the presence of 30 carbon signals and also showed two olefinic signals at δ 144.2 and 122.4, and two oxgenated carbon signals at δ 79.0 and 69.7 respectively. Additionally, the EIMS spectrum showed a typical fragment ion peak at *m/z* 234 due to a *retro* Diels Alder cleaveage occurring on the C ring of a 12-oleanene type triterpene (Herath *et al.*, 1998; Kagawa *et al.*, 1998). From these results, compound **4** indicated to be a dihydroxy oleanane type triterpene. The structure of **4** was determined to be 3β,28-dihydroxyolean-12-ene (erythrodiol) on the basis of the above evidences, together with a comparison of the above data with those reported in the literature (Nes *et al.*, 1981; Xue *et al.*, 1988).

Compound **5** was obtained as a white amorphorous powder from MeOH, and showed positive result in Liebermann-Burchard test. The EIMS of **5** showed an [M$^+$] ion at *m/z* 456. The IR spectrum exhibited a hydroxy (3420 cm^{-1}) absorption band. ^{13}C-NMR spectrum of **5** was very similar to that of **4**, suggesting the same skeleton. The main difference was the presence of δ 200.2 ppm resonance instead of δ 23.5 ppm of **4** in ^{13}C-NMR spectrum which can be assigned to carbonyl carbon (C-

11). On the basis of the above evidences, the structure of **5** was determined to be 3β,28-dihydroxy-11-oxoolean-12-ene (11-oxo-erythrodiol), together with a comparison of the above data with those published in the literature (Kagawa *et al.*, 1988).

Compound **6** was obtained as a white amorphorous powder from MeOH, and showed positive result in Liebermann-Burchard test. The EIMS of **6** showed an [M^+] ion at *m/z* 456. The IR spectrum exhibited a hydroxy (3510 cm^{-1}) absorption band. NMR spectra of **6** was very similar to that of **1**, suggesting the same skeleton. The main difference was the presence δ 181.8 ppm resonance instead of δ 18.0 ppm of **1** in ^{13}C-NMR spectrum which can be assigned to carbonyl carbon (C-28). On the basis of the above evidences, the structure of **6** was determined to be 3β-hydroxylup-20(29)-en-28-oic acid (betulinic acid), together with a direct comparison of the above data with those published in the literature (Cole *et al.*, 1991; Ikuta *et al.*, 1988; Otsuka *et al.*, 1981).

Lupeol (**1**), betulone (**2**), erythrodiol (**4**) and 11-oxo-erythrodiol (**5**) were isolated for the first time from *I. macropoda*.

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