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Triterpenoids of *Ilex Pubescens*

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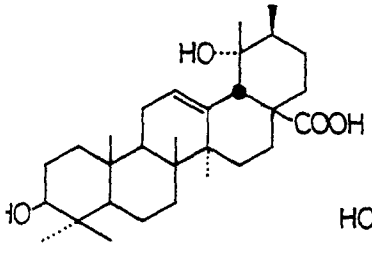
Abstract □ Four triterpenoids were isolated from the roots of *Ilex pubescens:* two new triterpenoids named pubescenolic acid (**I**) and pubescenic acid (**II**), and two known triterpenoids ilexolic acid (**III**) and oleanolic acid (**IV**). Chemical and spectroscopic studies have established **I** and **II** as 20-epipomolic acid and 24-carboxypomolic acid, repectively.

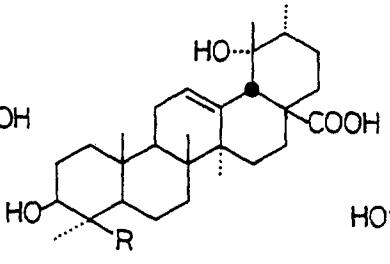
Keywords □ *Ilex pubescens,* Aquifoliaceae, triterpene, 20-epipomolic acid, pubescenolic acid, 24-carboxypomolic acid, pubescenic acid, ilexolic acid, oleanolic acid

The root of *Ilex pubescens* Hook. et Arn. Aquifoliaceae), mao-dong-quing (毛冬青), is videly used in China for the treatment of carliovascular disease, thromboangiitis obliterans, oronary disease, cerebral thrombosis, hyperholesteremia, *etc.*[1] The root is known to contain lavonoids glycosides[2,3] and ilexolide A (3-O-β-D-xylofuranosyl 18-dehydro-20-epiursolic acid).[4] These compounds were reported to be active principles of the plant.[1-4]

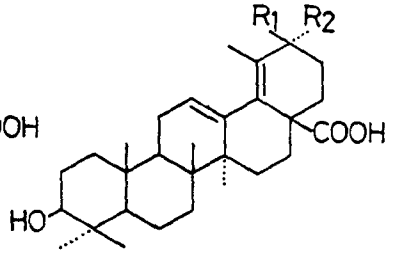
Our studies on the antithrombotic activities of the plant demonstrated that some of saponins, named ilexosides, were the active principles.[5] In the present communication we report the isolation and



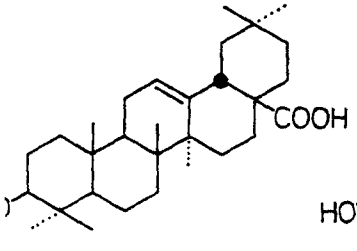
I



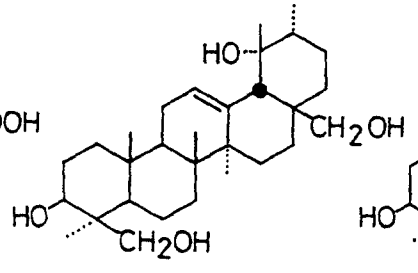
II : R=COOH
V : R=CH_3



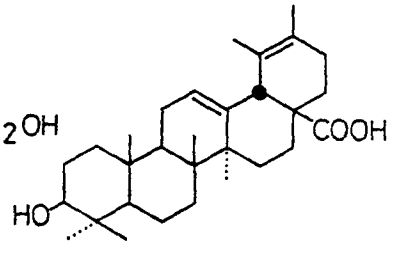
III : R_1=CH_3 , R_2=H
VII : R_1=H , R_2=CH_3



IV



VIII



VI

structure determination of two new triterpenoids in addition to the characterization of two known triterpenoids from the crude saponins of the root.

EXPERIMENTAL METHODS

General procedure

Acetylation was performed with Ac_2O/ pyridine at room temp. and methylation with diazomethane. Deacetylation of acetates was carried out in 0.1M K_2CO_3-85% MeOH solution overnight. Mps were determined on a Mitamura Ricken apparatus and are uncorrected. IR absorption spectra were obtained in KBr pellets on a Perkin-Elmer Model 281B spectrophotometer and optical rotations were obtained on a Rudolph Autopol III autometic polarimeter. CD/ORD spectra were obtained on a JASCO J-20 spectrometer. A recording spectrophotometer, Gilford Type 2600 was used for the measurements of UV/visible absorption spectra. NMR spectra were taken at 25 °C using tetramethylsilane (TMS) as an internal standard on a Varian Model FT 80A spectrometer (80MHZ) or a Nicolet NT-360 spectrometer. EIMS spectra were obtained on a Hewlett-Packard GC/MS spectrometer (Type 5985B). GLC was performed by a Hewlett-Packard Model 5985A GC chromatograph.

Extraction and isolation of triterpenoids

Dried roots (7.5 kg) were crushed and extracted with MeOH (50*l* × 4). The extracts were concentrated to dryness (1.8 kg), and then partitioned into 1-BuOH. BuOH extracts were evaporated to dryness (110g), and then hydrolized with 5% NaOH in 50% EtOH for 4hrs on a boiling water bath. After cooling, the hydrolysate was neutralized to pH 3 with d-H_2SO_4, and then extracted with 1-BuOH. The BuOH extract (50g) was roughly chromatographed over silica gel column using an eluting solvent of $CHCl_3$/MeOH/H_2O (30:10:1). Fractions showing positive reaction by Liebermann-Buchard reagent were pooled up and were freed from solvent. The residue (25g) was dissolved in MeOH (500 m*l*), and $HIO_4 \cdot 2H_2O$ (15g) in 100 m*l* H_2O was added over a period of lhr, while the solution was being stirred and cooled in an ice bath. The solution was allowed to stand in the dark at room temp. for two days, and an excess water (2*l*) was added. A pricipitate was taken by centrifugation, and suspended in 250 m*l* water. While the suspension was being stirred, potassium iodide (1g) was added and then sodium arsenite until iodine color disappeared. An equal volume of 10% KOH in EtOH was added. The solution was refluxed on a boiling water bath under nitrogen for 4 hrs, and carefully acidified with d-H_2SO_4 to about pH 3. Ethanol was removed *in vacuo*, extraction with EtOAc gave triterpenoids. The EtOAc extract was chromatographed over silica gel using an eluting solvent of hexane/ EtOAc (2:1) to be divided into three fractions: fr.1 (Rf 0.51), fr.2 (Rf 0.44) and fr.3 (Rf 0.08). Recrystallization of fr.2 from $CHCl_3$ afforded compound **I** (fine needles, 700 mg). Further chromatography of fr.3 over silica gel using the solvent of $CHCl_3$/MeOH (6:1) gave a compound with Rf 0.25. It was recrystallized from MeOH to yield compound **II** (600 mg). Repeated fractional recrystallization of fr.1 using CH_2Cl_2/MeOH (1:5) gave colorless needles (300 mg) (insoluble component in MeOH) (compound **III**). From the more soluble part in MeOH was obtained colorless needles (100 mg) (compound **IV**).

Pubescenolic acid (I)

mp: 256-258° ($CHCl_3$); $[\alpha]_D^{23}$: +0.48 (c=1, THF); IR (cm^{-1}, KBr): 3620, 3480(OH), 1690(COOH), 1030, 995(C-OH), 930(tert. C-OH); ^{1}H-NMR ($CDCl_3$/pyridine-d_5 1 drop) δ ppm: 0.66 (2 × 3H, s, 2 × CH_3), 0.75, 0.87(2 × 3H, each s, 2 × CH_3), 0.88(3H, d, J=6.7Hz, $C_{30}-H_3$), 1.06 (3H, s, $C_{29}-H_3$), 1.18(3H, s, $C_{27}-H_3$), 2.79(1H, s, $C_{18}-H$), 3.09(1H, t-like, C_3-H), 5.23 (1H, m, $C_{12}-H$); MS see Table II.

Acetylpubescenolic acid (Ia)

mp: 284-286° (amorphous); IR(cm^{-1}, KBr): 3510(OH), 3370, 1690(COOH), 1718, 1265 ($OCOCH_3$); ^{1}H-NMR($CDCl_3$, TMS) δ ppm: 0.74, 0.94, 1.18, 1.25(4 × 3H, each s, 4 × CH_3), 0.86(6H s, 2 × CH_3), 0.99(3H, d, J=7Hz, $C_{30}-H_3$), 2.04 (3H, s, $OCOCH_3$), 2.78(1H, br. s, $C_{18}-H$) 4.45(1H, t-like, C_3-H), 5.37(1H, m, $C_{12}-H$); MS *m/z* (Rel. Int. %): 514(M^+, 10.5), 468(44.7) 454(25.8), 396(15.7), 190(56.3), 146(100)

Pubescenolic acid methylester (Ib)

mp: 118-120° (MeOH); $[\alpha]_D^{23}$: +0.48($CHCl_3$) IR(cm^{-1}, KBr): 3620, 3520, 3460(OH), 172(($COOCH_3$), 1030, 995(C-OH); ^{1}H-NMR see Tabl I. ^{13}C-NMR see Table III. MS see Table II.

Acetylpubescenolic acid methylester(Ic)

mp: 220-222° (amorphous); $[\alpha]_D^{23}$: +0.3 ($CHCl_3$); IR(cm^{-1}, KBr): 3500(OH), 173(1718(ester); ^{1}H-NMR see Table I.

Pubescenic acid (II)

mp > 320° (MeOH); $[\alpha]_D^{23}$: + 0.672 (C = 0.

THF); UV: end absorption only (MeOH); IR (cm^{-1}, KBr): 3565, 3480(OH), 1695(COOH), 1020, 995(C-OH), 930(tert. C-OH); ^{1}H-NMR ($CDCl_3$ 1 pyridine-d_5 1 drop, TMS) δ ppm: 0.85, 0.93, 1.24, 1.33, 1.48(5 × 3H, each s, 5 × CH_3), 0.94(3H, d, J = 7.0Hz, $C_{30}-H_3$), 2.68(1H, s, $C_{18}-H$), 3.31(1H, dd, J = 4.4 and 11.2Hz, C_3-H), 5.40(1H, m, $C_{12}-H$); MS see Table II.

Acetylpubescenic acid (IIa)

mp: 318-320° (MeOH); $[\alpha]_D^{23}$: +0.208(c = 0.5, $CHCl_3$); IR(cm^{-1}, KBr): 2500-3600, 1700(COOH), 1740, 1250($OCOCH_3$); ^{1}H-NMR($CDCl_3$, TMS) δ ppm: 0.73, 0.86, 1.21(3 × 3H, each s, 3 × CH_3), 1.26(6H, s, 2 × CH_3), 0.94(3H, d, J = 7.OHz, $C_{30}-H_3$), 2.06 (3H, s, $OCOCH_3$), 2.55(1H, s, $C_{18}-H$), 4.55 (1H, dd, J = 3.8 and 12.4Hz, C_3-H), 5.33(1H, m, C_{12}-H); MS *m/z*(Rel. Int. %): 544(M^+, 7.6), 498(100), 426(41.5), 246(19.2), 220(36.8), 146(68.9)

Pubescenic acid methylester (IIb)

mp: 196-198°(MeOH); IR(cm^{-1}, KBr): 3500(OH), 1730, 1710($COOCH_3$), 1025, 995 (C-OH); ^{1}H-NMR see Table V. ^{13}C-NMR: see Table III. MS see Table II.

Acetylpubescenic acid methylester (IIc)

mp: 246-248° (MeOH); IR(cm^{-1}, KBr): 3510(OH), 1725, 1260, 1235($OCOCH_3$); ^{1}H-NMR see Table V. MS *m/z*(Rel. Int. %): 572(M^+, 4.7), 512($M^+ - CH_3COOH$, 50), 234(32.9), 179(100), 146(44.7)

Ilexolic acid (III)

mp: 161-163° (CH_2Cl_2 + MeOH); UV: 224.5nm (ε = 10,000, MeOH); IR(cm^{-1}, KBr): 3480(OH), 1695(COOH), 1650(C=C-H); ^{1}H-NMR($CDCl_3$, TMS) δ ppm: 0.78, 0.90(2 × 3H, each s, 2 × CH_3), 0.99(9H, s, 3 × CH_3), 1.04(3H, d, J = 7.0Hz, $C_{30}-H_3$), 1.73(3H, s, -C=C-CH_3), 3.23(1H, t-like, C_3-H), 5.41(1H, m, $C_{12}-H$); MS *m/z* (Rel. Int. %): 454(M^+, 100), 439($M^+ - CH_3$, 5.1), 409 (M^+ - COOH, 13.5), 201(45.1)

Ilexolic acid methylester (IIIb)

mp: 214-216° (MeOH); IR(cm^{-1}, KBr): 3450(OH), 1730(ester); ^{1}H-NMR see Table VI. MS *m/z*(Rel. Int. %): 468(M^+, 100), 453($M^+ - CH_3$, 5.7), 409($M^+ - COOCH_3$, 35.6), 201(41.4)

Acetylilexolic acid methylester (IIIc)

mp: 176-178° (MeOH); $[\alpha]_D^{23}$: +1.68 (c = 0.52, $CHCl_3$); UV: 229.5nm (ε = 13000, MeOH); IR(cm^{-1}, KBr): 1735, 1245 ($OCOCH_3$); ^{1}H-NMR see Table VI. ^{13}C-NMR see Table VII. MS *m/z*(Rel. Int. %): 510(M^+, 100), 495(M^+ -CH_3, 5.7), 451(M^+-$COOCH_3$, 39.8), 450(11.8), 201(42.2).

Oleanolic acid (IV)

mp: 308-310° (CH_2Cl_2/MeOH); IR(cm^{-1}, KBr): 3400(OH), 1700(COOH); ^{1}H-NMR($CDCl_3$, TMS) δ ppm: 0.75, 0.76, 0.97, 1.12(4 × 3H, each s, 4 × CH_3), 0.91(9H, s, 3 × CH_3), 2.79(1H, dd, J = 4.4 and 13.2Hz, $C_{18}-H$), 3.25(1H, t-like, C_3-H), 5.27(1H, m, C_{12}-H); MS *m/z*(Rel. Int. %): 456(M^+, 1.6), 248(100), 203(77.8), 208(4.5), 189(17.5)

Oleanolic acid methylester (IVb)

mp: 201-202° (MeOH); IR(cm^{-1}, KBr): 3400(OH), 1725($COOCH_3$); ^{1}H-NMR($CDCl_3$, TMS) δ ppm: 0.72, 0.77, 0.98, 1.12(4 × 3H, each s, 4 × CH_3), 0.90(9H, s, 3 × CH_3), 2.86(1H, dd, J = 4.87, and 13.6Hz, $C_{18}-H$), 3.22(1H, t-like, C_3-H), 3.61(3H, s, $COOCH_3$), 5.28(1H, m, C_{12}-H); MS *m/z* (Rel. Int. %): 470(M^+, 2.9), 262(60.0), 203(100), 208(3.4), 189(22.8)

Chromium trioxide-acetic acid oxidation of Ic

Compound **Ic** (160 mg) in acetic acid (2 m*l*) and dioxane (5 m*l*) was treated with chromium trioxide (450 mg) in acetic acid (8 m*l*). The mixture was stirred at room temperature for 5hrs and poured into ice water. A precipitate was extracted with $CHCl_3$. The extract was washed with water, dried over anhydrous Na_2SO_4, and freed from solvent. The residue was chromatographed on a silica gel column eluting with hexane/EtOAc (5: 1), and crystallized from hexane/$CHCl_3$ to give **Id** (50 mg). mp: 254-256° (amorphous); $[\alpha]_D^{11}$: +0.44(c = 1.0, CH_2Cl_2); UV: 252.5nm (ε= 7,800, MeOH); IR (cm^{-1}, KBr): 3460(OH), 1730, 1270($OCOCH_3$), 1710($COOCH_3$), 1660 (α,β-unsaturated ketone), 1625 (olefin); ^{1}H-NMR see Table I. ^{13}C-NMR see Table IV. MS *m/z* (Rel. Int. %): 542(M^+, 44.1), 483($M^+ - CO_2 - CH_3$, 100), 333(26.8), 292(4.1), 277(29.6), 255(15.2), 175(14.0), 119(14.6)

Alkaline hydrolysis of Id

A solution of **Id** (50 mg) in MeOH (6 m*l*) was treated with 0.7M K_2CO_3 (1 m*l*) + 1N NaOH (0.7 m*l*). The mixture was kept at room temperature overnight, neutralized with d-H_2SO_4 and extracted with $CHCl_3$. The extract was dried over anhydrous Na_2SO_4 and freed from solvent. The residue was chromatographed on a silica gel column eluting with hexane/EtOAc (1: 1) to yield **If**. **If** was

crystallized from $CHCl_3/Et_2O$ (10 mg). mp: 140-142°; $[\alpha]_D^{21}$: + 1.00(c = 0.17, MeOH); UV: 244.5nm (ε = 10000, MeOH); IR(cm^{-1}, KBr): 3460(OH), 1730($OCOCH_3$), 1715($COOCH_3$), 1700(Ketone), 1670(α,β-unsaturated ketone), 1635 (olefin); ^{1}H-NMR see Table I. ^{13}C-NMR see Table IV. MS *m/z*(Rel. Int. %): 500(M^+, 84.0), 441(M^+ -CO_2 -CH_3, 100), 333(62.6), 255(51.1), 235(36.4), 175(33.2), 119(20.7)

Acid hydrolysis of ilexoside D and ziyu-glycoside II

A mixture of ilexoside D[5] (300 mg) and 4N-HCl/dioxane/benzene (3:1:2, v/v, 30 m*l*) was refluxed for 4hrs. Solvents were removed under vacuo. The residue was diluted with water and a precipitate was taken up with EtOAc. The extract was chromatographed over silica gel eluting with hexane/EtOAc (5:1). A fraction having Rf 0.17 (100 mg) was obtained. GLC of an aliquot (5 mg) of the product was carried out as described below. Methylation of the remainder with diazomethane followed by acetylation with Ac_2O/pyridine afford a methyl ester acetate (100 mg). Fractional recrystallization using EtOAc/MeOH (1:2) gave colorless needles (70 mg). The substance was assigned as acetylilexolic acid methyl ester based on the physical data comparison with **IIIc**. A mixture of ziyu-glycoside II[6,7] and 4N HCl/dioxane/benzene (3:1:2, v/v, 120 m*l*) was refluxed for 4hrs. After the work-up described above, a fraction having Rf 0.17 (2g) was obtained. An aliquot (5 mg) of the hydrolysate was taken for GLC. Methylation of the remainder with diazomethane followed by acetylation with Ac_2O/pyridine afforded a methyl ester acetate (2.03g). By the method of Yosioka *et al.* [7]methyl 3-O-acetyl tomentosolic acid (**VIc**) (470 mg) and methyl 3-O-acetylvanguerolic acid (**VIIc**) (410 mg) were obtained.

Acetyltomentosolic acid methylester (VIc)

mp: 224-6° (MeOH/EtOAc = 2:1); IR(cm^{-1}, KBr): no hydroxyl, 1730, 1245(ester); ^{1}H-NMR ($CDCl_3$, TMS) δ ppm: 0.82, 0.95, 0.97(3 × 3H, each s, 3 × CH_3), 0.85(6H, s, 2 × CH_3), 1.62, 1.64(2 × 3H, each s, 2 × − C=C-CH_3), 2.03(3H, s, $OCOCH_3$), 3.20(1H, br. s, C_{18}-H), 3.63(3H, s, $COOCH_3$), 4.50(1H, t-like, C_3-H), 5.47(1H, t-like, C_{12}-H); Ms *m/z*(Rel. Int. %): 510(M^+,89.1), 495(M^+-CH_3, 3.4), 451(M^+-$COOCH_3$, 16.9), 247(100), 215(27.1), 201(36.8), 187(40.4)

Acetylvanguerolic acid methylester (VIIc)

mp: 176-178° (MeOH/EtOAc = 2:1); UV: 224.5nm (ε = 11,000, MeOH); IR(cm^{-1}, KBr): no hydroxyl, 1730, 1245(ester); ^{1}H-NMR see Table VI. ^{13}C-NMR see Table VII. MS *m/z*(Rel. Int. %): 510(M^+, 83), 495(M^+-CH_3, 3.1), 451(M^+-$COOCH_3$, 19.4), 247(100), 215(27.6), 201(35.8), 187(37.9)

GLC and GC/MS of hydrolysates of ilexoside D and ziyu-glycoside II

Each hydrolysate (5 mg) of ilexoside D[5] and ziyu-glycoside II[6] was methylated with diazomethane. And then the methyl ester in pyridine (0.5 m*l*) was treated with HMDS (100 u*l*) and TMS (50 u*l*). GLC was carried out under the following condition: SE-54 fushed silica gel column (0.2 mm i.d. × 12m), injection temp, 300°; FID temp, 300°; Aux temp, 200°; temp. 1, 180° (rate 4°/min); temp. 2, 280°; helium $\bar{\mu}$ = 17.4 cm/sec.

GC chromatogram of TMS-ether from the hydrolysate of ilexoside D showed two peaks with the ratio of 9 to 1 at Rt 29.9 (peak 1) and 31.9 min (peak 3), which corresponded to the TMS-ether of ilexolic acid methyl ester (**IIIb**) and the TMS-ether of vanguerolic acid methyl ester (**VIIb**), respectively.

GC chromatogram of TMS-ether from the hydrolysate of ziyu-glycoside II showed two peaks with the ratio of 1 to 1 at Rt 30.4 (peak 2) and 31.9 min (peak 3), which corresponded to the TMS-ether of tomentosolic acid methyl ester (**VIb**) and the TMS-ether of **VIIb**, respectively.

GC mass ion chromatograms of them were obtained under the same GLC condition mentioned above (Fig. 3).

Reduction of IIc with $LiAlH_4$

A solution of **IIc** (100 mg) in THF (3 m*l*) was dropped to a supension of $LiAlH_4$ (0.3 g) in THF (2 m*l*), while the suspension was being stirred at room temp. under nitrogen. After stirring for 5hrs, the reaction was stopped by addition of saturated NH_4Cl solution-ice. The product was diluted with water, acidified with d-HCl to pH 3, and taken up with EtOAc. The EtOAc extract was washed with water, dried over anhydrous Na_2SO_4 and freed from solvent. The residue was chromatographed over silica gel using $CHCl_3$/MeOH (10:1). Recrystallization of a substance (Rf 0.24) yielded **VIII** (30 mg).

Pubescentetraol (VIII)

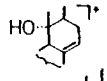
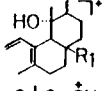
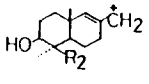
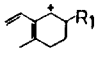
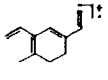
mp: 245-247° (MeOH); $[\alpha]_D^{23}$: + 0.278(c = 0.42, $CHCl_3$); IR(cm^{-1}, KBr): 3400 (OH), 1020 (C-O-C); ^{1}H-NMR ($CDCl_3$) see table V. MS *m/z* (Rel. Int. %): 474 (M^+, 2.2), 456 (M^+-H_2O, 37.6), 425 (18.8), 232 (13.1), 201 (28.5), 175 (32.0), 160 (100), 119 (23.7)

Table I. ^{1}H-NMR data of the derivatives of pubescenolic acid (I) and pomolic acid (V)

	Ib*	Vb*	Ic	Vc	Id	Vd	If	Vf
CH_3 23	0.99,s	0.99,s	0.86,s	0.87,s	0.86,s	0.86,s	0.99,s	0.99,s
24	0.78,s	0.78,s	0.86,s	0.87,s	0.86,s	0.86,s	0.81,s	0.81,s
25	0.91,s	0.91,s	0.92,s	0.94,s	1.12,s	1.11,s	1.18,s	1.18,s
26	0.69,s	0.68,s	0.69,s	0.68,s	0.86,s	0.86,s	0.99,s	0.99,s
27	1.25,s	1.25,s	1.25,s	1.25,s	1.48,s	1.48,s	1.26,s	1.26,s
29	1.17,s	1.21,s	1.17,s	1.22,s	1.21,s	1.22,s	2.11,s	2.11,s
30	1.00,d (J = 7.0Hz)	0.95,d (J = 7.2Hz)	0.99,d (J = 7.0Hz)	0.93,d (J = 7.2Hz)	1.00,d (J = 7.0Hz)	0.94,d (J = 7.2Hz)	1.09,d (J = 7.0Hz)	1.09,d (J = 7.0Hz)
C_3-H	3.20,dd (J = 4.8, 10.7Hz)	3.20,dd (J = 4.8, 11.6Hz)	4.51, t-like	4.51, t-like	4.51, t-like	4.51, t-like	3.21, t-like	3.21, t-like
C_9-H	—	—	—	—	2.44,s	2.44,s	2.28,s	2.28,s
C_{12}-H	5.33, t-like	5.35, t-like	5.32, t-like	5.34, t-like	5.61,s	5.64,s	5.64,s (J = 2.0Hz)	5.64,s (J = 2.0Hz)
C_{18}-H	2.83,s	2.60,s	2.82,s	2.58,s	2.91,s	2.72,s	2.59,q,H_B 2.80,q,H_A	2.59,q,H_B 2.80,q,H_A
$COOCH_3$	3.61,s	3.60,s	3.60,s	3.59,s	3.61,s	3.58,s	3.65,s	3.65,s
$OCOCH_3$	—	—	2.03,s	2.03,s	2.03,s	2.03,s	—	—

* Data by 360MHz NMR spectrometer

Table II. MS data of I, II, V and their methyl ester (relative intensity, %)

Fragments	I	V[6,8]	II	Ib	Vb[6,8]	IIb
M^+	472(16.7)	472(2.5)	502(11.1)	486(11.5)	486(3.1)	530(14.6)
M^+-H_2O	454(14.5)	454(4.4)	484(6.1)	468(13.6)	468(3.6)	512(8.9)
	426(48.0)	426(11.9)	456(73.7)	426(39.2)	426(13.0)	470(69.4)
	264(1.4)	264(6.9)	264(7.1)	278(0.4)	278(2.6)	278(1.9)
	207(32.3)	207(23.9)	237(4.0)	207(19.0)	207(19.2)	251(4.2)
	165(13.2)	165(11.9)	165(12.6)	179(100)	179(100)	179(100)
	146(100)	146(100)	146(100)	146(42.1)	146(55.4)	146(43.7)
	R_1 = COOH R_2 = CH_3	R_1 = COOH R_2 = CH_3	R_1 = COOH R_2 = COOH	R_1 = $COOCH_3$ R_2 = CH_3	R_1 = $COOCH_3$ R_2 = CH_3	R_1 = $COOCH_3$ R_2 = $COOCH_3$

Pubescentetraol acetate (VIIIa)

mp: 94-96° (MeOH); IR(cm^{-1}, KBr): 1735, 1235 ($OCOCH_3$), 1030 (C-O-C); ^{1}H-NMR ($CDCl_3$ see Table V. MS *m/z* (Rel. Int. %): 600 (M^+, 0.1), 541 (M^+ -CH_3COO, 3.8), 481 (2.4), 442 (4.1), 421 (2.1), 201 (31.6), 160 (100), 119 (32.5)

RESULTS AND DISCUSSION

The root of *Ilex pubescens* contains at least 23 kinds of saponins.[5] Smith's degradation of alkaline hydrolysate of crude saponins yielded four kinds of aglycones.

Compound **I**, $C_{30}H_{48}O_4$ (M^+ at *m/z* 472), gave positive Liebermann-Burchard reaction. Its IR spectrum showed the presence of OH (ν max 3620 and 3480) and COOH (ν max 1690) groups. It formed a monoacetate (**Ia**), a monomethyl ester (**Ib**) and a methyl ester acetate (**Ic**), each of which indicated the one OH group in **I** to be secondary and equatorial by an axial proton at 4.45 (t-like), 3.20 (dd,J = 4.8 and 10.7 Hz) and 4.51 (t-like) respectively in its NMR spectra (Table I). The mass spectrum of **I** showed the fragment peak at *m/z* 264, which is characteristic of retro Diels-Alder cleavage of olean-12-en-or urs-12-en-oic acid with a hydroxyl group on the D or E ring. The mass fragmentation patterns of **I** and its methyl ester (**Ib**) were nearly identical with those of pomolic aicd (**V**) and its methyl ester (**Vb**)[8] (Table II).

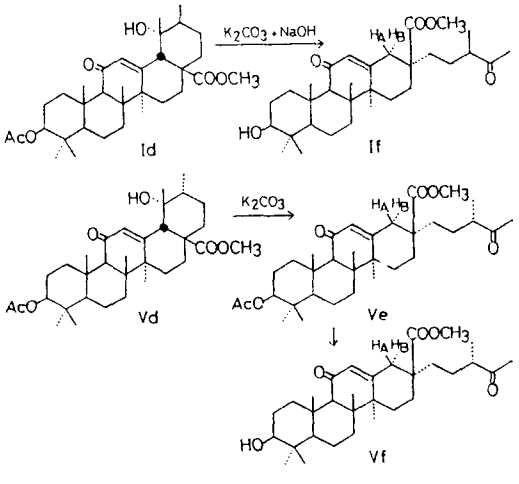


Fig. 1. E-ring opening of 11-keto-acetylpubescenolic acid methyl ester (Id) and 11-keto-acetylpomolic acid methyl ester (Vd) afforded 11,19-diketo-18,19-seco-20-epi-ursolic acid methyl ester (If) and 11,19-diketo-18,19-secoursolic acid methyl ester (Vf), respectively.

Table III. ^{13}C-NMR data of pubescenolic acid methylester(Ib), pomolic acid methylester(Vb) and pubescenic acid methylester(IIb) ($CDCl_3$, 90 MHz)

Carbon	Ib	Vb	IIb
1	38.4	38.5	39.2
2	27.2	27.2	28.1
3	78.9	79.0	78.3
4	38.7	38.7	49.0
5	55.1	55.1	56.5
6	18.4	18.4	20.2
7	32.7	32.8	33.0
8	39.8	39.9	39.7
9	47.2	47.2	46.6
10	37.0	37.0	37.3
11	23.6	23.7	23.8
12	128.4	129.2	129.1
13	137.7	138.0	138.3
14	41.1	41.1	41.1
15	28.1	28.2	28.1
16	26.0	25.5	25.5
17	47.4	47.9	47.9
18	46.6	53.2	53.3
19	73.9	73.1	73.1
20	41.4	41.1	41.1
21	23.9	26.0	26.0
22	31.2	37.4	37.3
23	28.1	28.1	23.6
24	15.6	15.6	178.4[a]
25	15.2	15.2	13.1
26	16.7	16.6	16.5
27	24.4	24.5	24.2
28	178.3	178.2	178.3[a]
29	30.0	27.4	27.4
30	15.7	16.1	16.1
$C_{28}OOCH_3$	51.6	51.5	51.5
$C_{24}OOCH_3$	—	—	51.1

a, Assignment may be reversed.

As the ^{1}H-NMR data of **Ib** were compared with those of methyl pomolate (**Vb**), the difference is that in the spectrum of **Ib**, the C_{18}-H signal at δ 2.83(s) was shifted downfield by 0.23 ppm, the C_{29}-H_3 signal at δ 1.17(s) upfield by 0.04 ppm and the C_{30}-H_3 signal at δ 1.00(d,J = 7.0Hz) downfield by 0.05 ppm (Table I). Other signals of **Ib** were nearly identical with those of **Vb**. The ^{13}C-NMR

Table IV. ^{13}C-NMR data of Id, Vd, If and Vf ($CDCl_3$, 20MHz)

Carbon	Id	Vd	If	Vf
1	38.0	38.0	39.3	39.3
2	23.0	23.5	27.3	27.3
3	80.7	80.6	78.7	78.6
4	38.8	38.8	39.1	39.1
5	55.1	55.1	55.2	55.2
6	17.4	17.4	17.5	17.5
7	33.2	33.2	33.9	33.9
8	43.6	43.5	43.5	43.5
9	61.4	61.2	61.2	61.2
10	37.1	37.0	37.2	37.1
11	199.7	199.8	199.5	199.5
12	132.5	133.0	126.9	126.9
13	163.3	163.3	161.2	161.3
14	45.7	45.7	44.1	44.1
15	28.5	28.6	27.8	27.8
16	25.8	25.2	30.4	30.4
17	47.3	47.7	47.9	47.9
18	47.1	53.8	27.0	27.0
19	74.1	73.1	211.5	211.5
20	42.6	41.4	47.1	47.0
21	23.7	25.8	30.7	30.7
22	30.6	36.7	38.8	38.7
23	28.1	28.1	28.1	28.1
24	16.6	16.6	15.6	15.6
25	16.1	16.1	16.3	16.3
26	18.8	18.7	18.6	18.6
27	21.1	21.1	19.8	19.8
28	177.5	177.4	175.3	175.3
29	29.8	27.1	28.1	28.1
30	15.6	15.8	16.4	16.4
$COOCH_3$	51.7	51.7	51.6	51.6
$OCOCH_3$	170.7	170.6	—	—
$OCOCH_3$	21.4	21.4	—	—

spectrum of **Ib** was also similar to that of **Vb**,[9] and the A to D rings carbon signals of both were essentially identical, although the signals of C_{18}, C_{21} and C_{22} in ring E were shifted to high field (Table III).

Chromium trioxide oxidation of **Ic** yielded a 11-keto derivative (**Id**). The ^{1}H-and ^{13}C-NMR data of **Id** were compared with those of 11-keto-pomolic acid methyl ester acetate (**Vd**) (Table I and IV). The C_{27}-H_3 signal at δ 1.48 of **Vd** was shifted downfield

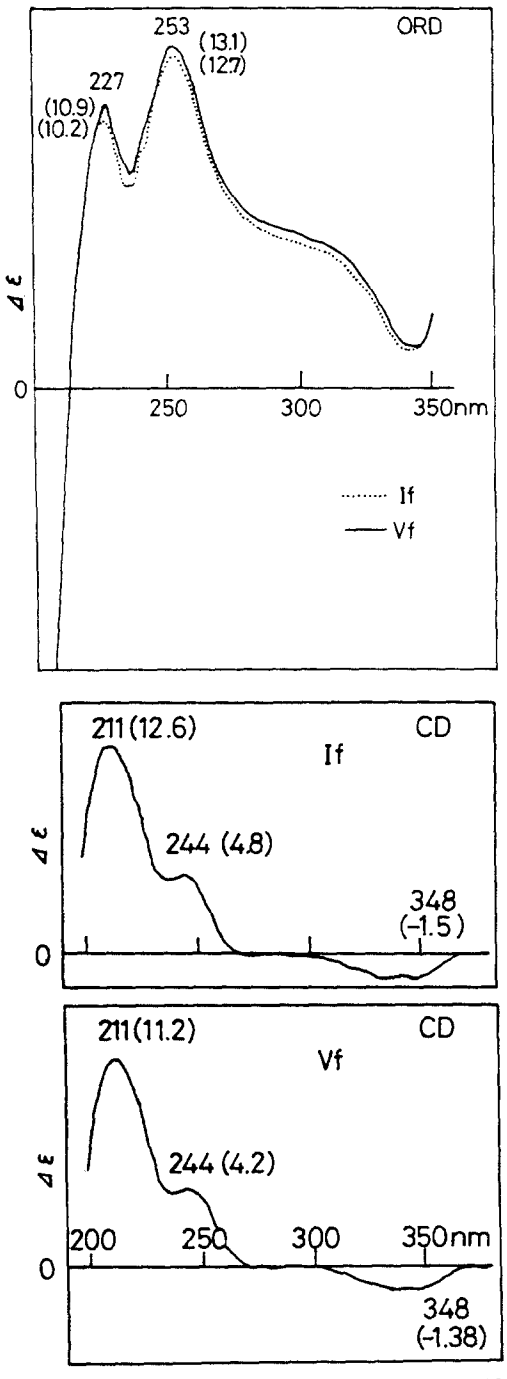


Fig. 2. ORD/CD spectra of 11,19-diketo-18,19-seco-20-epi-ursolic acid methyl ester (If) and 11,19-diketo-18,19-secoursolic acid methyl ester (Vf).
Solvent: CH_3CN. Concentration: i) ORD: **If**, 4.6×10^{-5}M, $[M]_{253} = 5322$; **Vf**, $4.9 = 10^{-5}$M, $[M]_{253} = 5347$, ii) CD: **If**, 1.38×10^{-4}M; **Vf**, 1.47×10^{-4}M.

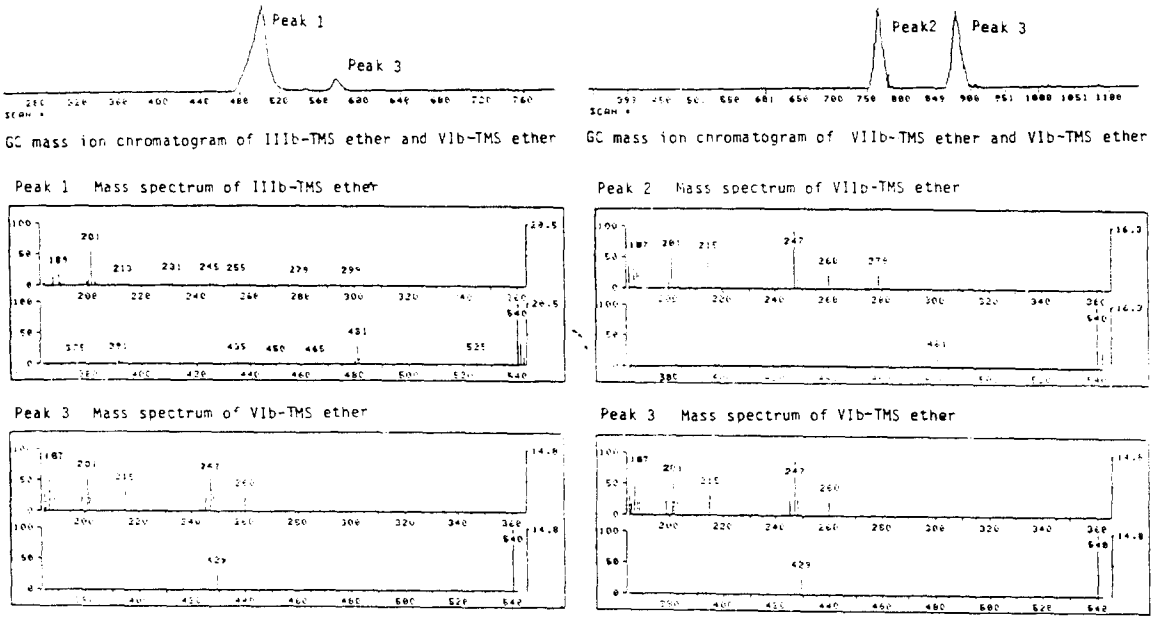


Fig. 3. GC mass ion chromatograms and mass spectra of the hydrolysates of ilexoside D (left) and Ziyu-glycoside II (right). Each hydrolysate was treated with diazomethane, and was trimethylsilylated. The data indicated that peak 1 corresponded to ilexolic acid methyl ester (**IIIb**)-TMS ether, peak 2 vanguerolic acid methyl ester (**VIIb**)-TMS ether, and peak 3 tomentosolic acid methyl ester (**VIb**)-TMS ether.

by 0.23 ppm compared with that at 1.25 of **Vc**, and the same chemical shift was also observed in **Id**, revealing that the configuration of C_{19}-tert. OH of **I** is the same α as **V**[10] (Table I). Really, the ^{13}C-NMR signal at δ 21.1 of C_{27} of **Id** was identical with that of **Vd** (Table IV). We already reported that mild alkaline hydrolysis of **Vd** induced the E-ring opening due to retrograde aldol condensation to yield **Ve**, and then **Ve** was deacetylated to give **Vf**.[6] Alkaline treatment of **Id** also induced its E-ring opening to yield **If** without giving its acetate. The PMR, CMR, CD and ORD spectra of **If** were all superimposed of those of **Vf**, although the C_{30} configuration of **If** was expected to be reverse to that of **Vf** (Table I, IV and Fig. 1,2). However, these data clearly indicated that the stereochemistry of A to D rings of **I** was identical with that of **V**. Thus, **I** must be the epimer of pomolic acid (**V**) in the ring E.

For the purpose of establishing the configuration of the E ring of **I**, ileoxside D,[5] a diglycoside of **I**, was hydrolized with HCl to obtain dehydrated products. It is known that acid hydrolysis of ziyu-glycoside II, 3-O-α-L-arabopyranosyl-pomolic acid, gave tomentosolic acid (**VI**) and vanguerolic acid (**VII**).[7] We have also obtained **VI** and **VII** as their methyl ester acetate (**VIc** and **VIIc**), and the ratio of the products was proved to be 1 : 1 by GLC analysis of their TMS-ether methyl esters (Fig. 3b). There are two situations for dehydration of C_{19}-OH in **V**; one is between C_{19}-OH and C_{18}-H, and the other between C_{19}-OH and C_{20}-H. Since both of C_{19}-OH/C_{18}-H and C_{19}-OH/C_{20}-H are *trans*, acid hydrolysis of ziyu-glycoside II afforded the same ratio of the artifacts. On the other hand, the hydrolysate of ilexoside D turned out to be composed of ilexolic acid (**III**) (described below) and **VI** with the ratio of 9 to 1, indicating that the configuration of C_{19}-OH/C_{20}-H must be *cis* since dehydration predominates at *trans* position (Fig. 3a). Ilexolic acid (**III**) was proved to be 20-epivanguerolic acid (see below). Thus, compound **I** was established as 20-epipomolic acid, and we named it pubescenolic acid. In the ^{13}C-NMR data of **Ib**, the high field shifts of C_{18} and C_{22} of **Ib** by 6.6 and 6.2 ppm compared with those of **Vb** can be explained in terms of the γ-gauche effect of the C_{30}-H_3 (axial, β) of **Ib**.

Compound **II**, $C_{30}H_{46}O_6$ (M^+ at m/z 502), gave positive Liebermann-Burchard reaction. Its IR spectrum indicated the presence of OH (λ max 3565, 3480, 995, 930) and COOH (λ max 1695) groups. **II** formed a monoacetate (**IIa**), a dimethyl ester (**IIb**) and a dimethyl ester monoacetate (**IIc**), each of which showed the one OH group in **II** to be secondary and equatorial by an axial proton at δ

Table V. ^{1}H NMR data of the derivatives of pubescenic acid (II) ($CDCl_3$)

	IIb**	IIc	VIII	VIIIa
C_{23}-H_3	1.42,s	1.23,s	1.24,s	1.00,s
C_{24}	—	—	3.34, 4.20(2H, ABq, J = 11.1 Hz)	4.10, 4.37(2H, ABq, J = 11.4 Hz)
C_{25}-H_3	0.75,s	0.79,s	0.89,s	0.96,s
C_{26}-H_3	0.70,s	0.67,s	0.94,s	0.96,s
C_{27}-H_3	1.26,s	1.22,s	1.29,s	1.27,s
C_{28}	—	—	3.17, 3.47(2H, ABq, J = 10.7 Hz)	3.60, 4,01(2H, ABq, J = 10.7 Hz)
C_{29}-H_3	1.20,s	1.22,s	1.42,s	1.14,s
C_{30}-H_3	0.94,d (J = 7.0Hz)	0.93,d (J = 7.0Hz)	*	*
C_3-H	3.08,ddd (J = 4.4, 11.7, 11.7Hz)	4.53,dd (J = 4.3, 11.6Hz)	3.20,m	4.61,m
C_{12}-H	5.34, t-like	5.34, t-like	5.25,m	5.24,m
C_{18}-H	2.59,s	2.59,s	—	—
$COOCH_3$	3.60,s	3.58,s	—	—
	3.67,s	3.65,s		
$OCOCH_3$	—	2.05,s	—	2.02,s(6H) 2.03,s(3H)

* Mixed with another peaks. ** Data by 360MHz NMR spectrometer.

4.55, 3.08 and 4.53 respectively in its ^{1}H-NMR spectra (Table V). The mass spectrum of **II** showed the fragment peak at *m/z* 264, which is characteristic of retro Diels-Alder cleavage of olean-12-en- or urs-12-en-oic acid with a hydroxyl group on the D or E ring, and the same peaks at *m/z* 165 and 146 (a base peak) as those of **V** (Table II). The ^{13}C-NMR spectrum of **IIb** showed 32 carbon signals, *i.e.* CH_3-×6, -CH_2-×4, >C<×5, >CH-O×1, ≡C-O×1, >C=CH-×1, CO×2, -OCH_3×2 (Table III). The chemical shifts of C,D and E ring carbons of **IIb** were essentially identical with those of methyl pomolate (**Vb**). Another COOH group must be located at C_4, of which signal was shifted to down field by 10.3 ppm. In the case of ^{13}C-NMR of gypsogenic aicd,[11] 23-carboxyoleanolic acid, its carbon signals in A ring were much different from those of **IIb.** All of the above data suggested **II** to be 24-carboxypomolic acid. Reduction of **IIc** with $LiAlH_4$ afforded a tetraol ($C_{30}H_{50}O_4$, M^+ at *m/z* 474), named pubescentetraol (**VIII**). Acetylation of **VIII** with Ac_2O/pyridine yielded a triacetate (**VIIIa**). The ^{1}H-NMR spectra of **VIII** and **VIIIa** were compared with those of soyasapogenol A, hederagenin and their acetates.[12] In the ^{1}H-NMR of **VIII,** one couple of AB quartet at δ 3.34 and 4.20 (J = 11.1Hz) can be assignable to C_{24}-$\mathbf{H_2}$ (OH), and the other at δ 3.17 and 3.47 (J = 10.7Hz) to C_{28}-$\mathbf{H_2}$ (OH). In the ^{1}H-NMR of **VIIIa,** one couple of AB quartet at δ 4.10 and 4.37 (J = 11.4Hz) must correspond to C_{24}-$\mathbf{H_2}$ (OAc), and the other at δ 3.60 and 4.01 (J = 10.7Hz) to C_{28}-$\mathbf{H_2}$ (OAc) (Table V). Therefore, **II** can be formulated as 3β, 19α-dihydroxyurs-12-en-24,28-dioic acid, *i.e.* 24-carboxypomolic aicd, and we named it pubescenic acid.

Compound **III,** $C_{30}H_{46}O_3$ (M^+ at *m/z* 454), gave positive Liebermann-Burchard reaction. Its IR spectrum indicated the presence of OH (λ max 3480), COOH (λ max 1695) and olefin (λ max 1650) groups. It UV spectrum showed the presence of conjugated double bond (224.5 nm, ε = 10,000). **III** formed a methyl ester (**IIIb**) and a methyl ester acetate (**IIIc**), each of which showed the OH group in **III** to be secondary and equatorial by an axial proton at δ 3.21 and 4.52 respectively in its ^{1}H-NMR spectra (Table VI). The ^{1}H-NMR spectra showed signals due to seven tertiary methyls, among which one methyl splitted in two at δ 1.02 (J = 7.0Hz) and another signal at δ 1.70 can be assignable to -C=C-CH_3. The mass spectra of **III, IIIb** and **IIIc** showed the base M^+ peak, the fragment peaks of [M^+-CH_3] and [M^+-COOR] and the

Table VI. ^{1}H-NMR data of the derivatives of ilexolic acid (III) and vanguerolic acid (VII) ($CDCl_3$)

		IIIb	VIIb	IIIc	VIIc
CH_3	23	0.98,s	0.98,s	0.86,s	0.86,s
	24	0.78,s	0.78,s	0.86,s	0.86,s
	25	0.87,s	0.87,s	0.86,s	0.86,s
	26	0.98,s	0.99,s	0.98,s	0.99,s
	27	0.98,s	0.96,s	0.98,s	0.96,s
	29	1.70,s	1.72,s	1.70,s	1.72,s
	30	1.02,d	1.07,d	1.02,d	1.07,d
		(J = 7.0Hz)	(J = 7.0Hz)	(J = 7.0Hz)	(J = 7.0Hz)
C_3-H		3.21, t-like	3.21, t-like	4.52, t-like	4.52, t-like
C_{12}-H		5.32, t-like	5.35, t-like	5.31, t-like	5.35,t-like
$COOCH_3$		3.60	3.60	3.60	3.60
$OCOCH_3$		—	—	2.04	2.04

Table VII. ^{13}C-NMR data of acetylilexolic acid methylester (IIIc) and acetylvanguerolic acid methylester (VIIc) ($CDCl_3$, 20 MHz)

Carbon	IIIc	VIIc	Carbon	IIIc	VIIc
1	38.8	38.9	18	135.6[a]	135.8[a]
2	23.7	23.7	19	133.9[a]	133.0[a]
3	80.9	80.9	20	36.9	34.3
4	39.1	39.1	21	34.3	31.2
5	55.6	55.6	22	34.3	34.5
6	18.2	18.3	23	28.2	28.2
7	34.8	35.1	24	16.7	16.8
8	37.7	37.8	25	16.0	16.1
9	47.8	47.9	26	17.6	17.8
10	36.9	36.9	27	22.0	21.7
11	23.2	23.2	28	177.0	176.8
12	126.1	125.6	29	19.9[b]	19.2[b]
13	138.9	138.7	30	20.1[b]	18.8[b]
14	44.4	44.7	$COOCH_3$	51.3	51.4
15	28.7	28.6	$OCOCH_3$	170.7	170.7
16	28.3	26.6	$OCOCH_3$	21.1	21.1
17	50.0	49.5			

a,b, Assignments may be reversed.

fragement peak at m/z 201, which are characteristic of olean-12-ens or urs-12-ens containing a secondary double bond in E-ring.[13] The ^{1}H- NMR spectra of **IIIb** and its acetate (**IIIc**) were very similar to those of methyl vanguerolate (**VIIb**) and its acetate (**VIIc**), rspectively, except the C_{30}-H_3 signal (Table VI). As the ^{13}C-NMR spectrum of **IIIc** was compared with that of **VIIc**, the carbon signals in A to C rings of both compounds were nearly identical, although some carbon signals in D and E rings were slightly different (Table VII). All the data indicated **III** to be 20-epivanguerolic acid. Major component of acid hydrolysate of ilexoside D was found to be identical with compound **III** in all respects (TLC, mass, IR and NMR). Thus, **III** was established as ilexolic acid.[4]

Compound **IV**, C_{30} H_{48} O_3 (M^+ at m/z 456), gave positive Liebermann-Burchard reaction. It formed a monomethyl ester (**IVb**). Their IR, ^{1}H-NMR and mass spectra were superimposed on those of oleanolic acid and its methyl ester.

After the publication of "Doctral Thesis of Sookmyung Women's University (December 1986)" of Dr. S.K. Baik, one of the authors of this article, a paper of new triterpene saponins from *Ilex* pubescens [*Chem. Pharm. Bull.* **35**, 524 (1987)] was published by Hidaka, K. *et al.* They isolated two triterpenes, named ilexgenins A and B, which correspond to pubescenic acid (**II**) and pubescenolic acid (**I**), repectively, in this article.

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