Arch. Pharm. Res. 10(2), 132-141 (1987)

New Triterpenoid Saponins from *Ilex pubescens*

Yong Nam Han, Seung Kyung Baik*, Tae Hee Kim* and Byung Hoon Han

Natural Products Research Institute, Seoul National University, Seoul 110 and
**College of Pharmacy, Sookmyung Women's University, Seoul 151, Korea*

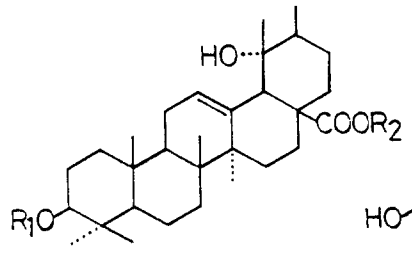
(Received April 28, 1987)

Abstract□New triterpenoid saponins, ilexosides A,-D,-E,-H,-J,-K and -O, have been isolated from the root of *Ilex pubescens*. Chemical and spectroscopic studies have established their structures as shown in formulae **1,2,8,11,3,4,** and **5.**

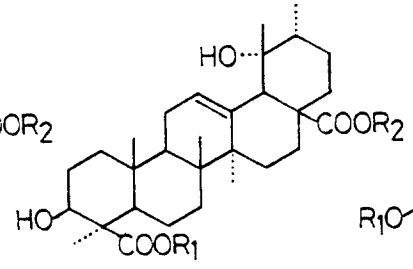
Keywords□*Ilex pubescens,* Aquifoliaceae, saponin, triterpenoid, ^{13}C-NMR, ilexolic acid, pubescenolic acid, pubescenic acid, secondary ion mass.

The root or leaf of *Ilex pubescens* Hook. et Arn (Aquifoliaceae), "Maodongquing" (毛冬青), is widely used in China for the treatment of cardiovascular diseases, cerebral thrombosis, *etc.*[1] The plant is known to contain homovanillic acid, vomifoliol[2], 3,4-dihydroxyacetophenone, 6-methyl-7-hydroxycoumarin, 6,7-dihydroxycoumarin[3], glaberide I, scopoletin, esculetin,[4-6] flavonoid glycosides,[7,8] and ilexolide A.[9,10] The latter two kinds of compounds have been isolated from the root, and the remainders are the constituents from the leaf. All these compounds were reported to be the active constituents of the plant.[1-10]

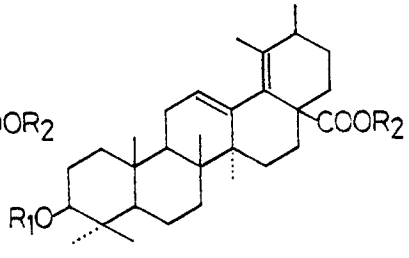
The antithrombotic activities of some medicinal plants were screened, utilizing the measurement of bleeding and clotting times.[11-14] Among them, *Ilex pubescens* root was shown to prolong the bleeding time three times as compared with a control group.[14] And the methanol extract of the root strongly inhibited the generation of malondialdehyde, which is known to be concomitantly released with thromboxane A_2 during thrombin-induced aggregation of rat platelets. By solvent fractionation, Sephadex LH-20 and silica gel column chromatography, saponin components were found to be the active principles.[14] Seven kinds of saponins, named ilexosides A,-D,-E,-H,-J,-K and-O, were purely isolated from the BuOH fraction of the plant. This communication describes the structural elucidation of the seven saponins. Their



	R_1	R_2
1	-Xyl	Glu
2	-Xyl^2-Glu	H
3	-Xyl^2-Glu^2-Rha	H
4	-Xyl^2-Glu	Glu
5	-Xyl^2-Glu^2-Rha	Glu
6	-Xyl	H
7	H	H



	R_1	R_2
8	H	Glu
8b	CH_3	Glu
9	H	H
9b	CH_3	CH_3
10	CH_3	H



	R_1	R_2
11	-Xyl^2-Glu	Glu
12	-Xyl^2-Glu	H
13	H	H

Xyl: β-D-xylopyranosyl
Glu: β-D-glucopyranosyl
Rha: α-L-rhamnopyranosyl

Chart 1

aglycones were reported in a separate article.[15]

Structures of ilexosides A,D,J,K and O

By Smith's degradation, ilexosides A(**1**), -D(**2**), -J(**3**), -K(**4**), and -O(**5**) were proved to have the same aglycone, pubescenolic acid (**7**),[15] which is supported by their ^{13}C-NMR data as shown in Table I. The sugar compositions of the saponins were determined by GLC of their acid hydrolysates as shown in Table III, and the mole ratio of sugars were also confirmed by the ^{13}C-NMR analysis (Table II).

Ilexoside A(**1**) is composed of xylose, glucose and **7**. Its IR spectrum indicated the presence of OH (3400cm^{-1}) and ester (1735cm^{-1}) groups. By ^{1}H-NMR analysis, the sugars were identified as β-D-xylopyranose (anomeric H at δ 4.28,d, J = 6.7Hz) and β-D-glucopyranose (anomeric H at δ 5.33,d, J = 6.7Hz). The absolute configurations of the sugars were also ascertained to be both D, by analogy with methyl β-D-xylopyranoside and methyl β-D-glucopyranoside[16] (Table II).

Acetylation of **1** yielded a peracetate (**1a**). From

Table I. ^{13}C-NMR data for genin moieties of Ilexosides A(1),-D(2),-J(3),-K(4) and -O(5), and their genin methyl ester(7b) (δ in pyridine-d_5, 20MHz)

Carbon	1	2	3	4	5	7b
1	39.1	39.1	39.1	39.1	38.9	38.4
2	26.8	26.8	26.9	26.8	26.6	27.2
3	89.0	89.1	89.8	89.2	90.0	78.9
4	39.7	39.7	39.8	39.7	39.8	38.7
5	56.2	56.2	56.3	56.1	56.3	55.1
6	18.8	18.8	18.9	18.9	18.8	18.4
7	33.5	33.8	33.7	33.6	33.5	32.7
8	40.6	40.5	40.5	40.5	40.6	39.8
9	47.9	47.9	47.9	47.9	47.9	47.2
10	37.2	37.2	37.3	37.1	37.2	37.0
11	24.1	24.1	24.2	24.1	24.2	23.6
12	127.8	127.5	127.5	127.9	127.9	128.4
13	138.9	139.5	139.6	138.7	138.9	137.7
14	42.3	42.3	42.3	42.2	42.3	41.1
15	29.4	29.4	29.4	29.4	29.3	28.1
16	27.0	27.2	27.3	27.1	27.0	26.0
17	48.5	48.1	48.1	48.4	48.5	47.4
18	47.3	47.6	47.6	47.3	47.4	46.6
19	73.7	73.7	73.7	73.7	73.7	73.9
20	42.9	43.1	43.1	42.6	42.8	41.4
21	24.7	25.1	25.0	24.7	24.7	23.9
22	31.9	32.5	32.6	31.8	31.8	31.2
23	28.4	28.3	28.6	28.3	28.6	28.1
24	17.6	17.4	17.5	17.5	17.6	15.6
25	15.8	15.7	15.7	15.8	15.7	15.2
26	17.1	16.9	16.9	16.8	16.9	16.7
27	24.4	24.5	24.5	24.4	24.4	24.4
28	177.2	180.8	180.8	177.2	177.2	178.3
29	29.9	29.9	29.9	29.9	29.8	30.0
30	16.2	16.3	16.4	16.2	16.2	15.7
$C_{28}OOCH_3$	—	—	—	—	—	51.6

*Cited from reference 15. Measured in $CDCl_3$.

Table II. ^{13}C-NMR data for sugar moieties of ilexosides A(1),-D(2),-J(3),-K(4) and -O(5), (δ in pyridine-d_5, 20MHz)

Methylglycoside*		1	2	3	4	5
β-D-Xylose						
1	106.1	107.4	105.6	105.8	105.4	105.8
2	74.6	75.4	83.1	79.5	82.6	79.4
3	78.1	78.3	78.2	77.7	77.8	77.7
4	70.9	71.2	71.0	71.3	70.9	71.3
5	66.9	66.9	66.6	66.7	66.6	66.5
β-D-Glucose						
1	105.4		105.8	102.3	105.6	102.4
2	74.8		76.8	79.4	76.5	79.2
3	78.1		78.1	78.8	77.8	78.9
4	71.4		71.9	72.7	71.7	72.9
5	78.1		78.1	78.8	77.8	78.9
6	62.5		62.9	63.4	62.7	63.4
α-L-Rhamnose						
1	102.4			101.9		101.9
2	71.9			72.3		72.4
3	72.5			72.6		72.6
4	73.6			74.3		74.3
5	69.4			69.5		69.5
6	18.4			18.9		18.9
β-D-Glucose, C_{28}						
1	105.4	95.9			95.7	95.8
2	74.8	74.1			73.9	74.2
3	78.1	78.9			78.7	78.8
4	71.4	71.4			71.3	71.4
5	78.1	78.9			78.7	78.8
6	62.5	62.5			62.5	62.5

*Cited from reference 16.

Table III. Sugar compositions of ilexosides A(1),-D(2), -E(8),-H(11),-J(3),-K(4) and -O(5), and prosapogenins A(6) and -H(12)

	sugars	ratio
1	xylose/glucose	1:1
2	xylose/glucose	1:1
8	glucose	—
11	xylose/glucose	1:2
3	xylose/glucose/rhamnose	1:1:1
4	xylose/glucose	1:2
5	xylose/glucose/rhamnose	1:2:1
6	xylose	—
12	xylose/glucose	1:1

the ^{1}H-NMR spectrum of **1a**, the sugars were reconfirmed as β-D-glucopyranose (anomeric H at δ 4.50,d, J = 6.6Hz) and β-D-glucopyranose (anomeric H at δ 5.56,d, J = 7.5Hz).

Alkaline hydrolysis of **1** afforded prosapogenin A (**6**), of which sugar was proved to be xylose alone (Table III). The IR spectrum of **6** revealed the presence of free COOH (1690cm^{-1}). Methylation of **6** gave a methyl ester (**6b**). In the ^{1}H-NMR spectrum of **6**, the anomeric proton of xylose was exhibited at δ 4.33 (d, J = 6.7Hz). Therefore, the glucose moiety of **1** should be located at C_{28} of **7** as an ester, which was reconfirmed by the anomeric carbon signal appeared at δ 95.9 (ester glycosidic glucose) in the ^{13}C-NMR spectrum of **1** (Table II). For clarification of the location of the xylose moiety, the ^{13}C-NMR of **1** was inspected. The carbon signals were essentially identical with those of pubescenolic acid (**7**) except those due to the A ring carbons (Table I). Among the A ring carbon signals, the C-3 signal was significantly shifted downfield (glycosidation shift,[17] 10.1ppm), impling that the xylose molecule was bound through glycoside linkage to the C-3 hydroxyl group of the aglycone (**7**). Thus, the structure of **1** was established as the 3-O-β-D-xylopyranosyl-28-O-β-D-glucopyranoside of **7**.

Ilexoside D (**2**) is composed of xylose, glucose and **7**. It was shown to have the molecular formula C_{41} H_{66} O_{13} from the results of SI mass[18] [m/z 773 (M + Li)$^+$ and 779 (M + 2Li-H)$^+$]. Its IR spectrum indicated the presence of free COOH group (1690cm^{-1}), suggesting that no sugar was linked at C-28 of **7**. Methylation of **2** with diazomethane afforded a monomethyl ester (**2b**), acetylation of **2** did a peracetate (**2a**), and methylation of **2a** did a monomethyl ester peracetate (**2c**).

By ^{1}H-NMR analysis of **2**, the sugars were identified as β-D-xylopyranose (anomeric H at δ 4.39,d, J = 5.8Hz) and β-D-glucopyranose (anomeric H at δ 4.58,d, J = 6.9Hz). The results were also reconfirmed by the ^{1}H-NMR analysis of **2a** and **2c**. In the ^{13}C-NMR spectrum of **2**, the carbon signals were essentially identical with those of **7** except those due to the A ring carbons (Table I). Among them, the C-3 signal was significantly shifted downfield by 10.2ppm. In the ^{13}C-NMR data of the sugar moieties, the carbon signal of C-2 of xylose was notably shifted downfield by 8.5ppm compared with methyl β-D-xylopyranoside (Table II). These observations implied that glucose molecule was bound through glycoside linkage to the C-2 hydroxyl group of xylose, of which molecule was linked at the C-3 hydroxyl group of the aglycone (**7**). The sugar sequence was also ascertained by the results of SI mass [m/z 599 (M + 2Li-H-$C_6H_{12}O_6$)$^+$ and 467 (M + 2Li-H-$C_6H_{12}O_6$ -$C_5H_8O_4$)$^+$]. Therefore, the structure of **2** was established as the 3-O-β-D-glucopyranosyl (1→2) -β-D-xylopyranoside of **7**.

Ilexoside J (**3**) was shown to have the molecular formula C_{47} H_{76} O_{17} from the results of SI mass [m/z 919 (M + Li)$^+$ and 925 (M + 2Li-H)$^+$]. The mole ratio of xylose, glucose and rhamnose of **3** was proved to be 1:1:1 by GLC of its hydrolysate and ^{13}C-NMR analysis (Table III and II). Its IR spectrum showed the presence of free COOH group (1700cm^{-1}). Methylation of **3** with diazomethane afforded a monomethyl ester (**3b**). In the ^{13}C-NMR data of **3**, the C-3 signal of the aglycone moiety was significantly shifted by 10.9ppm compared with that of the aglycone (**7b**). These observations indicated that all the sugar molecules were located at C-3-O position of **7**.

In the ^{1}H-NMR of **3**, the anomeric protons of xylose, glucose and rhamnose appeared at δ 4.30 (d, J = 6.2Hz), 4.79 (d, J = 6.8Hz) and 5.18 (d, J = 0.9Hz), indicating that they were β-D-xylopyranose, β-D-glucopyranose and α-L-rhamnopyranose, respectively. Acetylation of **3** and **3b** yielded a acetate (**3a**) and a methyl ester acetate (**3c**), respectively. The ^{1}H-NMR analysis of the derivatives (**3a, 3b** and **3c**) confirmed the absolute configurations of the sugars.

The ^{13}C-NMR analysis of the sugar moieties of **3** revealed 2-linked xylopyranose (its C-2 signal at δ 79.5), 2-linked glucopyranose (its C-2 signal at δ 79.3) and terminal rhamnopyranose (it C-2 signal at δ 72.3). The sugar sequence was ascertained by the results of SI mass]m/z 773 (M + Li-$C_6H_{10}O_4$)$^+$ 617 (M + 2Li-H-$C_6H_{10}O_4$ -$C_6H_{10}O_5$)$^+$ and 465

$(M + 2Li\text{-}H\text{-}C_6H_{12}O_5\ \text{-}C_6H_{12}O_5\ \text{-}C_5H_8O_4)^+]$. Thus, the structure of **3** was established as the 3-O-α-L-rhamnopyranosyl (1→2)-β-D-glucopyranosyl (1→2)-β-D-xylopyranoside of **7**.

Ilexoside K (**4**) contained xylose and glucose with the mole ratio of 1 to 2 (Table II and III). Its IR spectrum indicated the presence of ester group (1735cm^{-1}). The ^{13}C-NMR spectrum of **4** was very similar to that of **2** except the C-28 signal at δ 177.2 which was shifted upfield by 3.6ppm compared with the C-28 signal of **2** (Table I), and the esterified β-D-glucopyranosyl signals (its C-1 at δ 95.7) (Table II).

In the ^{1}H-NMR spectrum of **4**, three anomeric proton signals were observed at δ 4.44 (d, J = 6.0Hz, β-D-xylopyranose), 4.67 (d, J = 6.9Hz, one β-D-glucopyranose) and 5.57 (d, J = 7.0Hz, the other esterified β-D-glucopyranose). The anomeric protons were detected in the ^{1}H-NMR of ilexoside K acetate (**4a**). Alkaline hydrolysis of **4** afforded ilexoside D (**2**). Therefore, the structure of **4** was established as the 28-O-β-D-glucopyranoside of **2**.

Ilexoside O (**5**) involved xylose, glucose and rhamnose with the ratio of 1:2:1 (Table II and Table III). Its IR spectrum showed an ester band (1730cm^{-1}). The ^{13}C-NMR spectrum of **5** was very similar to that of **3**. The difference was observed in the C-28 signal shifted upfield by 3.6ppm and the esterified β-D-glucopyranosyl signals (Table I and II). In the ^{1}H-NMR spectrum of **5**, four anomeric proton signals were observed at δ 4.31 (d, J = 6.7Hz, β-D-xylopyranose), 4.80 (d, J = 6.8Hz, one B-D-glucopyranose), 5.18 (d, J = 1.0Hz, α-L-rhamnopyranose) and 5.34 (d, J = 6.8Hz, the other esterified β-D-glucopyranose). The anomeric protons were also observed in that of ilexoside O acetate (**5a**). Alkaline treatment of **5** yielded ilexoside J (**3**). Thus, the structure of **5** was established as the 28-O-β-D-glucopyranoside of **3**.

Structure of ilexoside E (8)

Ilexoside E (**8**) gave positive Liebermann-Burchard reaction. Its IR spectrum showed the presence of OH (3400cm^{-1}), ester (1725cm^{-1}) and

Table IV. ^{13}C-NMR data of ilexoside Eb (8b), pubescenic acid dimethyl ester (9b), prosapogenin H(12) and acetylilexolic acid methyl ester (13c)

Carbon	9b*	8b**	13c*	12**
1	39.2	39.0	38.9	39.1
2	28.1	28.0	23.7	26.6
3	78.3	78.3	80.9	88.7
4	49.0	49.0	39.1	39.4
5	56.5	56.2	55.6	56.0
6	20.2	20.1	18.3	18.3
7	33.0	33.0	34.9	35.4
8	39.7	39.8	37.8	39.2
9	46.6	46.7	47.9	48.1
10	37.3	37.4	36.9	36.7
11	23.8	23.7	23.2	23.3
12	129.1	128.2	126.1	126.1
13	138.3	138.8	138.9	139.5
14	41.1	41.4	44.5	44.7
15	28.1	28.1	28.8	29.1
16	25.5	25.5	28.3	28.8
17	47.9	47.8	50.1	50.2
18	53.3	53.5	135.7	135.5
19	73.1	73.1	134.0	134.8
20	41.1	41.4	37.0	37.3
21	26.0	26.3	34.4	34.8
22	37.3	36.8	34.3	34.9
23	23.6	23.5	28.2	28.1
24	178.4	176.6	16.8	17.9
25	13.1	12.8	16.1	16.0
26	16.5	16.5	17.6	16.5
27	24.2	24.0	22.1	22.1
28	178.3	176.6	177.1	178.9
29	27.4	28.3	20.0	20.0
30	16.1	16.2	20.2	20.3
1′		95.8		105.4
2′		73.9		83.0
3′		78.7		78.1
4′		71.3		70.7
5′		78.7		66.4
6′		62.5		—
1″				105.7
2″				76.6
3″				77.7
4″				71.6
5″				77.7
6″				62.6
24OOCH$_3$	51.1	50.3		—
28OOCH$_3$	51.5	—		51.4
3-OCOCH$_3$	—	—		170.7
3-OCOCH$_3$	—	—		21.1

*Cited from reference 15. Measured in $CDCl_3$
**Measured in pyridine-d_5

COOH (1690cm^{-1}) groups. Alkaline hydrolysis of **8** yielded the aglycone, pubescenic acid (**9**).[15] From acid hydrolysate of **8**, only glucose was detected by TLC and GLC (Table III). Methylation of **8** with diazomethane afforded a monomethyl ester (**8b**), which was shown to have the molecular formula $C_{37}H_{58}O_{11}$ from the results of SI mass [*m/z* 685 $(M+Li)^+$ and 667 $(M-H_2O)^+$], indicating that **8** contained one mole of glucose. Acetylation of **8** with Ac_2O/pyridine gave a pentacetate (**8a**). In the ^{1}H-NMR spectrum of **8a**, the anomeric proton signal of glucose appeared at δ 5.80 (d, J = 7.6Hz), revealing it to be the esterified β-D-glucopyranose, which was reconfirmed by the anomeric carbon signal at δ 95.8 in the ^{13}C-NMR of **8b** (Table IV).

The ^{13}C-NMR data of **8b** was not informative for clarification of the location of the glucose moiety, because the carbon signals of the aglycone of **8b** were nearly identical with those of pubescenic acid dimethyl ester (**9b**).[15] Alkaline hydrolysis of **8b** afforded pubescenic acid monomethyl ester (**10**). On comparison of **10** with **9b** in their mass spectra, the differences of CH_2 mass units were found in the fragment peaks due to retro-Diels-Alder cleavage (*m/z* 264 of **10**; 278 of **9b**) followed by further fragmentation [*i.e.*, *m/z* 246 (264-H_2O); 260 (278-H_2O)], as well as in their molecular ion peaks (*m/z* 516; 530). The results indicated **10** to be 24-methyl ester of the aglycone. Thus, the glucose moiety should be located at C_{28}. Therefore, the structure of **8** was estblished as the 28-O-β-D-glucopyranoside of **9**.

Structure of ilexoside H (11)

Ilexoside H (**11**) exhibited UV absorption at 248nm (ε = 9,800). Its IR spectrum showed the presence of OH (3400cm^{-1}) and ester (1735cm^{-1}) groups. Smith's degradation of **11** afforded the aglycone, ilexolic acid (**13**). From acid hydrolysate, xylose and glucose with the ratio of 1 to 2 were detected by TLC and GLC (Table III). Acetylation of **11** gave a peracetate (**11a**). In the ^{1}H-NMR spectrum of **11a** three anomeric proton signals appeared at δ 4.49 (d, J = 6.2Hz, β-D-xylopyranose), 4.69 (d, J = 7.2Hz, β-D-glucopyranose) and 5.64 (d, J = 7.3Hz, esterified β-D-glucopyranose).

Alkaline hydrolysis of **11** yielded prosapogenin H (**12**), which also exhibited UV absorption at 247.5nm. The IR spectrum of **12** showed the presence of OH (3400cm^{-1}) and COOH (1700cm^{-1}) groups. The sugar composition of **12** was determined as xylose and glucose with the ratio 1 to 1 (Table III). Two anomeric proton signals appeared at δ 4.45 (d, J = 6.0Hz, β-D-xylopyranose) and 4.66 (d, J = 7.0Hz, β-D-glucopyranose) in the ^{1}H-NMR spectrum of **12**. The ^{13}C-NMR analysis, revealed 2-linked xylopyranose (C-2 of xylose at δ 83.0)and terminal glucopyranose (Table IV). Thus, the structure of 11 was established as the 3-O-β-D-glucopyranosyl (1→2)-β-D-xylopyranosido-28-β-D-glucopyranoside of **13**.

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**(2), 524 (1987)] was published by Hidaka, K. *et al.* They isolated four saponins, named ilexsaponins A_1, B_1 B_2 and B_3, which correspond to ilexosides E, D, J and K, respectively, in this article. They reported that ilexsaponin B_3 possessed an activity against experimental hypercholesteremia in mice.

EXPERIMENTAL METHODS

Plant materials

The root of *Ilex pubescens* was purchased from a marcket of Hong Kong in 1983.

General Procedures

Acetylation of a compound was performed with Ac_2O/pyridine at room temp. and methylation was carried out with diazomethane, in the usual ways. Mps were determined on a Mitamura-Ricken apparatus and are uncorrected. IR absorption spectra were obtained in KBr pellets on a Perkin-Elmer Model 283B spectrophotometer and optical rotations were obtained on a Rudolph Autopol III automatic polarimeter. A recording spectrophotometer, Gilford Type 2600 was used for the measurements of UV/visible absorption spectra. NMR spectra were taken at 25 °C using tetramethysilane (TMS) as an internal (80MHz). EIMS spectra were obtained on a Hewlett Packard GC/MS spectrometer (Type 5985B) and SIMS spectra on a Hitachi M-80 high resoultion mass spectrometer. GLC was performed by a Hewlett-Packard Model 5985A GC chromatograph.

Isolation of ilexosides

Dried roots (30kg) were crushed and extracted with MeOH (120*l* X 3), and the extracts were concentrated to dryness (7.7kg). A portion of the methanol extract (1kg) was suspended in water and then partitioned into 1-butanol. The BuOH extract was evaporated to dryness (440g).

A portion of the BuOH extract (110g) was chromatographed on a column of silica gel (E. Merck,

70-230 mesh) and eluted with $CHCl_3/MeOH/H_2O$ (80:20:1→60:20: 2→60:30:4→ 30:20:5→10:10: 4), and divided into six fractions: fr.1 (14.4g), fr.2 (31.2g), fr.3 (20.2g), fr.4 (32.4g), fr.5(7.3g) and fr.6 (2.6g).

Fractions 2,3 and 4 were further chromatographed on silica gel using $CHCl_3/MeOH/H_2O$ and/or $EtOAc/MeOH/H_2O$ system, and on Sephadex LH-20 using MeOH eluent. From fr.2, ilexosides A (400mg), D (470mg) and E (2.62g) were purely obtained; from fr.3, ilexoside J (120mg); from fr.4, ilexosides H (250mg), K (3.0g) and O (300mg).

Smith's degradation of ilexosides

Each ilexoside (30mg) was dissolved in pyridine (1m*l*) and MeOH (5m*l*), and periodic acid dihydrate (50mg) in lm*l* MeOH was added over a period of 20min, while the solution was being stirred and cooled in an ice bath. The solution was allowed to stand in the dark at room temperature for two days, and 30m*l* water was added. A precipitate was taken by centrifugation, and suspended with 10m*l* water. Then while the suspension was being stirred, potassium iodide (10mg) was added and then sodium arsenite until iodine color disappeared. An equal volume of 10% potassium hydroxide in ethanol was added. The solution was heated on a boiling water bath under nitrogen for 4hrs, and carefully acidified with d-H_2SO_4 to about pH 3-4. Ethanol was removed *in vacuo*, and extraction with EtOAc gave sapogenin. From ilexoside A, D, J, K and O, pubescenolic acid (**7**) was obtained; from ilexoside E pubescenic acid (**9**); from ilexoside H ilexolic acid (**13**).

Pubescenolic acid (7)

mp: 256-258° (fine needles from $CHCl_3$); $[\alpha]_D^{23}$: + 0.48 (THF); IR (cm^{-1}): 3620, 3480 (OH), 1690 (COOH), 995 (C-OH), 930 (tert. C-OH); ^{1}H-NMR ($CDCl_3/C_6D_5N$): 0.66, 0.75, 0.87 (12H, 4 X 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, br. s, C_{18}-H), 3.09 (1H, t-like, C_3-H), 5.23 (1H, m, C_{12}-H); MS (m/z, %): 472 M^+, 16.7), 454 (M^+-H_2O, 14.5), 426 (M^+-HCOOH, 48.0), 354 (14.0), 264 (1.4), 207 (32.3), 190 (41.0), 146 (100)

Pubescenic acid (9)

mp>320 (needles from MeOH); $[\alpha]_D^{23}$+ 0.67 (c = 0.5%, THF); UV (MeOH): end absorption only; IR (cm^{-1}): 3565, 3480 (OH), 1695 (COOH), 995 (C-OH), 930 (tert. C-OH); ^{1}H-NMR ($CDCl_3/C_6D_5N$): 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, br.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 (m/z, %): 502 (M^+, 10.3), 456 (M^+ -HCOOH, 93.6), 384 (26.3), 220 (37.8), 201 (23.1), 146 (100)

Ilexolic acid (13)

mp: 161-3° (needles from $CH_2Cl_2/MeOH$); $[\alpha]_D^{23}$: + 1.9 (c= 1%, $CHCl_3$); IR (cm^{-1}): 3480 (OH), 1695 (COOH); ^{1}H-NMR ($CDCl_3$): 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, m, C_3 -H), 5.41 (1H, m, C_{12} -H); MS (m/z, %): 454 (M^+, 100), 439 (M^+ -CH_3, 5.1), 409 (M^+ -HCOOH, 13.5), 248 (15.7), 231 (22.8), 201 (45.1), 119 (13.5)

Ilexoside A (1)

mp: 212-214° (amorphous); $[\alpha]_D^{23}$: + 0.059 (c = 0.91%, THF); UV: end absorption only (MeOH); IR (cm^{-1}, KBr): 3400 (OH), 1735 (ester), 1100-1000 (glycoside); ^{1}H-NMR (CD_3 OD, TMS) δ ppm: 2.73 (1H, br. s, C_{18} -H), 4.28 (1H, d, J = 6.7Hz, anomeric H of D-xylose), 5.31 (1H, m, C_{12} -H), 5.33 (1H, d, J = 6.7Hz, anomeric H of C_{28} -D-glucose)

Ilexoside A acetate (1a)

mp: 136-138° (amorphous); IR (cm^{-1}, KBr): 3480 (OH), 1760, 1255 ($OCOCH_3$); ^{1}H-NMR ($CDCl_3$, TMS) δ ppm: 2.01 (6 × 3H, s, 6 × $OCOCH_3$), 2.05 (3H, s, 1 × $OCOCH_3$), 2.74 (1H, br. s, C_{18} -H), 3.07 (1H, m, C_3 -H) 4.50 (1H, d, J = 6.6Hz, anomeric H of D-xylose), 5.34 (1H, m, C_{12}-H), 5.56 (1H, d, J = 7.5Hz, anomeric H of C_{28}- D-glucose)

Ilexoside D (2)

mp: 264-266° (needles fro MeOH): $[\alpha]_D^{23}$: + 0.275 (c = 0.53%, THF); UV: end absorption only (MeOH); IR (cm^{-1}, KBr): 3400 (OH), 1690 (COOH), 1100-1000 (glycoside); ^{1}H-NMR (CD_3 OD/$CDCl_3$ = 1:1, TMS) δ ppm: 0.73, 0.79, 0.88, 1.00, 1.13 ,1.23 (6 × 3H, each s, 6 × CH_3), 0.96 (3H, d, J = 7.0Hz, C_{30}-H_3), 2.75 (1H, br.s, C_{18} -H), 3.05 (1H, m, C_3 – H), 4.39 (1H, d, J = 5.8Hz, anomeric H of D-xylose), 4.58 (1H, d, J = 6.9Hz, anomeric H of D-glucose), 5.27 (1H, m, C_{12} – H); ^{13}C-NMR see Table I and II. SI mass: *m/z* 779 $(M + 2Li-H)^+$, 773 $(M + Li)^+$, 727 $(M + Li-HCOOH)^+$, 599 $(M + 2Li-H-C_6H_{12}O_6)^+$, 467 $(M + 2Li-H-C_6H_{12}O_6-C_5H_8O_4)^+$

Ilexoside D acetate (2a)

mp: 169-171° (needles fromn MeOH); $[\alpha]_D^{23}$:

-0.014 (c = 2.0%, $CHCl_3$); IR (cm^{-1}, KBr): 3510 (OH): 1750, 1230 ($OCOCH_3$); ^{1}H-NMR ($CDCl_3$, TMS) δ ppm: 0.71, 0.77, 0.89, 1.00, 1.16, 1.23 (6×3H, each s, $6 \times CH_3$), 0.99 (3H, d, J = 7.0Hz, $C_{30}-H_3$), 1.97, 2.05, 2.09 (3×3H, each s, $3 \times OCOCH_3$), 2.01 (9H, s, $3 \times OCOCH_3$), 2.76 (1H, br.s, $C_{18}-H$), 3.06 (1H, m, C_3-H), 4.52 (1H, d, J = 6.0Hz, anomeric H of D-xylose), 4.68 (1H, d, J = 7.3Hz, anomeric H of D-glucose), 5.30 (1H, m, $C_{12}-H$)

Ilexoside D methylester (2b)

mp: 196-198° (needles from MeOH); IR (cm^{-1}, KBr): 3400 (OH), 1720 ($COOH_3$); ^{1}H-NMR ($CD_3OD/CDCl_3 = 1:1$, TMS) δ ppm: 0.70, 0.85, 0.94, 1.05, 1.16, 1.29 (6×3H, each s, $6 \times CH_3$), 1.00 (3H, d, J = 7.0 Hz, C_{30}-H_3), 2.77 (1H, br. s, C_{18}-H), 3.63(3H, s, $COOCH_3$), 5.30 (1H, m, C_{12}-H)

Ilexoside D methylester acetate (2c)

mp: 140-142° (needle from MeOH); IR (cm^{-1}, KBr): 1760, 1230 ($OCOCH_3$); ^{1}H-NMR ($CDCl_3$, TMS) δ ppm: 0.67, 0.78, 0.88, 1.00, 1.16, 1.23 ($3H \times 6$, each s, $6 \times CH$), 0.98 (3H, d, J = 7Hz, $C_{30}-H_3$), 1.97, 2.05, 2.09 (3×3H, each s, $3 \times OCOCH_3$), 2.00 (9H, s, $3 \times OCOCH_3$), 2.82 (1H, br.s, $C_{18}-H$), 3.06 (1H, m, C_3-H), 3.60 (3H, s, $COOCH_3$), 4.49 (1H, d, J = 6.4Hz, anomeric H of D-xylose), 4.68 (1H, J = 7.1Hz, anomeric H of D-glucose), 5.31 (1H, m, $C_{12}-H$)

Ilexoside J (3)

mp: 248-250 (MeOH); $[\alpha]_D^{23}$: +0.115(c = 0.27%, THF); UV: end absorption only; IR(cm^{-1}, KBr): 3400(OH), 1700(COOH), 1100-1000(glycoside); ^{1}H-NMR ($CD_3OD/CDCl_3 = 1:1$, TMS) δ ppm: 0.79, 0.85, 0.94, 1.08, 1.17, 1.29(6×3H, each s, $6 \times CH_3$), 0.99(3H, d, J = 7.0Hz, $C_{30}-H_3$), 1.26(3H, d, J = 5.3Hz, CH_3 of L-rhamnose), 2.75(1H, br.s, $C_{18}-H$), 4.30(1H, d, J = 6.2Hz, anomeric H of D-xylose), 4.79(1H, d, J = 6.8Hz, anomeric H of D-glucose), 5.18(1H, d, J = 0.9Hz anomeric H of L-rhamnose), 5.27(1H, m, $C_{12}-H$); ^{13}C-NMR see Table I and II; SI mass: *m/z* 925$(M + 2Li-H)^+$, 919$(M + Li)^+$, 873$(M + Li-HCOOH)^+$, 773$(M + Li-C_6H_{10}O_4)^+$, 617$(M + 2Li-H-C_6H_{10}O_4-C_6H_{10}O_5)^+$, 465$(M + 2Li-H-C_6H_{12}O_5-C_6H_{12}O_5-C_5H_8O_4)^+$

Ilexoside J acetate (3a)

mp: 169-171° (MeOH); $[\alpha]_D^{23}$: +0.10(c = 0.28%, $CDCl_3$); IR(cm^{-1}, KBr): 1755(ester), 1705(COOH); ^{1}H-NMR($CDCl_3$, TMS) δ ppm: 0.75, 0.81, 0.92, 1.03, 1.17, 1.25(6×3H, each s, $6 \times CH_3$), 1.00(3H, d, J = 7.0Hz, $C_{30}-H_3$), 1.20(3H, d, J = 5.3Hz, CH_3 of L-rhamnose), 1.96, 2.00, 2.01, 2.12 (4×3H, s, $4 \times COCH_3$), 2.04, 2.09(2×6H, s, $4 \times OCOCH_3$), 2.77(1H, br.s, $C_{18}-H$), 3.04(1H, m, C_3-H), 4.52(1H, d, J = 5.6Hz, anomeric H of D-xylose), 4.66(1H, d, J = 7.6Hz, anomeric H of D-glucose), 5.13(1H, d, J = 0.9Hz, anomeric H of L-rhamnose), 5.30(1H, m, $C_{12}-H$)

Ilexoside J methylester (3b)

mp: 204-206° (needles from MeOH); IR(cm^{-1}, KBr): 3400(OH), 1725, 1710(ester); ^{1}H-NMR ($CD_3OD/CDCl_3 = 1:1$, TMS) δ ppm: 0.75, 0.85, 0.94, 1.06, 1.17, 1.29(6×3H, each s, $6 \times CH_3$), 1.00(3H, d, J = 7.0Hz, $C_{30}-H_3$), 1.26(3H, d, J = 5.1Hz, CH_3 of L-rhamnose), 2.77(1H, br.s, $C_{18}-H$), 3.62(3H, s, $COOCH_3$), 4.83(1H, d, J = 6.9Hz, anomeric H of D-glucose), 5.21(1H, d, J = 0.9Hz, anomeric H of L-rhamnose), 5.27(1H, m, $C_{12}-H$)

Ilexoside J methylester acetate (3c)

mp: 141-143° (MeOH); IR(cm^{-1}, KBr): 1755, 1230($OCOCH_3$); ^{1}H-NMR($CDCl_3$, TMS) δ ppm: 0.68, 0.79, 0.89, 1.01, 1.17, 1.24(6×3H, each s, $6 \times CH_3$), 0.97(3H, d, J = 7.0Hz, $C_{30}-H_3$), 1.19(3H, d, J = 5.1Hz, CH_3 of L-rhamnose), 1.96, 1.99, 2.01, 2.12(4×3H, each s, $4 \times OCOCH_3$), 2.04, 2.09(2×6H, each s, $4 \times OCOCH_3$), 2.82(1H, br.s, $C_{18}-H$), 3.03(1H, m, C_3-H), 4.49(1H, d, J = 6.0Hz, anomeric H of D-xylose), 4.65(1H, d, J = 7.5Hz, anomeric H of D-glucose), 5.13(1H, br.s, anomeric H of L-rhamnose), 5.30(1H, m, $C_{12}-H$)

Ilexoside K (4)

mp: 204-206°;$[\alpha]_D^{23}$: +0.156(c = 0.57%, THF); UV: end absorption only (MeOH); IR(cm^{-1}, KBr): 3400(OH), 1735(ester), 1100-1000(glycoside); ^{1}H-NMR($CDCl_3/CD_3OD/D_2O/TFA = 7:3:0.5:1$, TMS) δ ppm: 0.74, 0.83, 0.93, 1.03, 1.26(6×3H, each s, $6 \times CH_3$), 0.99(3H, d, J = 7.0Hz, $C_{30}-H_3$), 2.80(1H, br.s, $C_{18}-H$), 4.44(1H, d, J = 6.0Hz, anomeric H of D-xylose), 4.67(1H, d, J = 6.9Hz, anomeric H of D-glucose), 5.57(1H, d, J = 8Hz, anomeric H of C_{28}-D-glucose), 5.23(1H, m, $C_{12}-H$); ^{13}CMR see Table I and II.

Ilexoside K acetate (4a)

mp: 148-150 (amorphous); IR(cm^{-1}, KBr): 1760, 1230($OCOCH_3$); ^{1}H-NMR($CDCl_3$, TMS) δ ppm: 0.70, 0.79, 0.90, 1.00, 1.17, 1.24(6×3H, each s, $6 \times CH_3$), 0.99(3H, d, J = 7.0Hz, $C_{30}-H_3$), 2.01(21H, s, $7 \times OCOCH_3$), 2.05(6H, s, $2 \times OCOCH_3$), 2.08(3H, s, $1 \times OCOCH_3$), 2.75(1H,

br.s, C_{18}-H), 3.06(1H, m, C_3-H), 4.49(1H, d, J=6.2Hz, anomeric H of D-xylose), 4.69(1H, d, J=7.3Hz, anomeric H of D-glucose), 5.57(1H, d, J=7.2Hz, anomeric H of C_{28}-D-glucose), 5.34(1H, m, C_{12}-H)

Ilexoside O (5)

mp: 224-226° (MeOH); $[\alpha]_D^{23}$: −0.194(c=1.0%, pyridine); UV: end absorption only(MeOH); IR(cm^{-1}, KBr): 3400(OH), 1730(ester), 1100-1000 (glycoside); ^{1}H-NMR($CD_3OD/CDCl_3$=1:1, TMS) δ ppm: 0.77, 0.84, 0.94, 1.07, 1.18, 1.29(6×3H, each s, 6×CH_3), 1.02(3H, d, J=7.0Hz, C_{30}-H_3), 1.26(3H, d, J=5.9Hz, CH_3 of L-rhamnose), 2.74(1H, br.s, C_{18}-H), 4.31(1H, d, J=6.7Hz, anomeric H of D-xylose), 4.80(1H, d, J=6.8Hz, anomeric H of D-glucose), 5.18(1H, d, J=1.0Hz, anomeric H of L-rhamnose), 5.34(1H, d, J=6.8Hz, anomeric H of C_{28}-D-glucose), 5.28(1H, m, C_{12}-H); ^{13}C-NMR see Table I and II

Ilexoside O acetate (5a)

mp: 147-149° (MeOH); IR(cm^{-1}, KBr): 1755, 1230($OCOCH_3$); ^{1}H-NMR($CDCl_3$, TMS) δ ppm: 0.72, 0.81, 0.92, 1.02, 1.17, 1.24(6×3H, each s, 6×CH_3), 0.99(3H, d, J=7.0Hz, C_{30}-H_3), 1.18(3H, d, J=5.6Hz, CH_3 of L-rhamnose), 2.01, 2.04, 2.08(12×$OCOCH_3$), 2.74(1H, br.s, C_{18}-H), 3.03(1H, m, C_3-H), 4.49(1H, d, J=5.8Hz, anomeric of D-xylose), 4.65(1H, d, J=6.7Hz, anomeric H of D-glucose), 5.13(1H, br.s, anomeric H of L-rhamnose), 5.56(1H, d, J=7.3Hz, anomeric H of C_{28}-D-glucose), 5.33(1H, m, C_{12}-H)

Ilexoside E (8)

UV: end absorption only (MeOH); IR(cm^{-1}, KBr): 3400(OH), 1725(ester), 1690(COOH), 1100-1000(glycoside); ^{1}H-NMR($CD_3OD/CDCl_3$=1:1, TMS) ppm: 0.77, 0.88, 1.18, 1.28, 1.37(5×3H, each s, 5×CH_3), 0.95(3H, d, J=7.0Hz, C_{30}-H_3), 2.52(1H, br.s, C_{18}-H), 3.10(1H, dd, J=4.8 and 11.9Hz, C_3-H), 5.32(1H, m, C_{12}-H), 5.32 (1H, d, J=6.6Hz, anomeric H of C_{28}-D-glucose)

Ilexoside E acetate (8a)

mp: 180-200° (needles from MeOH); $[\alpha]_D^{23}$: +0.241(c=1.0%, $CHCl_3$); IR(cm^{-1}, KBr): 3550(OH), 3500-2800(COOH), 1760, 1230 ($OCOCH_3$); ^{1}H-NMR($CDCl_3$, TMS) δ ppm: 0.69 (3H, s, 1×CH_3), 0.92(6H, s, 2×CH_3), 1.25(9H, s, 3×CH_3), 1.99, 2.02(2×6H, each s, 4×$OCOCH_3$), 2.06(3H, s, 1×$OCOCH_3$), 2.51(1H, br.s, C_{18}-H), 4.55(1H, dd, J=4.0 and 11.6Hz, C_3-H), 5.50(1H, m, C_{12}-H), 5.80(1H, d, J=7.6Hz, anomeric H of 28-D-glucose)

Ilexoside E methylester (8b)

mp: 197-199° (amorphous); $[\alpha]_D^{23}$: +0.393 (c=0.55%, THF); IR (cm^{-1}, KBr): 3450 (OH), 1730, 1705 (ester); ^{1}H-NMR ($CD_3OD/CDCl_3$=1:1, TMS) δ ppm: 0.78 (6H, s, 2×CH_3), 0.95 (3H, d, J=7.0Hz, C_{30}-H_3), 1.21, 1.30, 1.39 (3×3H, each s, 3×CH_3), 2.55 (1H, br.s, C_{18}-H), 3.12 (1H, dd, J=4.6 and 11.3Hz, C_3-H), 3.67 (3H, s, $COOCH_3$), 5.31 (1H, m, C_{12}-H), 5.35 (1H, d, J=7Hz, anomeric H of C_{28}-D-glucose); CMR: see Table I and II; SI mass: m/z 685 $(M+Li)^+$, 667 $(M-H_2O)^+$, 529 $(M+2Li-H-C_6H_{10}O_5)^+$, 523 $(M+Li-C_6H_{10}O_5)^+$, 477 $(M+Li-C_6H_{10}O_5-HCOOH)^+$

Ilexoside E methylester acetate (8c)

mp: 144-146 (needles from MeOH); $[\alpha]_D^{23}$: +0.304 (c=1.0%, $CHCl_3$); IR (cm^{-1}, KBr): 3560 (OH), 1760, 1230 ($OCOCH_3$); ^{1}H-NMR ($CDCl_3$, TMS) δ ppm: 0.70, 0.79 (2×3H, each s, 2×CH_3), 0.93 (3H, d, J=7.0Hz, C_{30}-H_3), 1.21 (6H, s, 2×CH_3), 1.22 (3H, s, 1×CH_3), 2.01 (3×3H, s, 3×$OCOCH_3$), 2.05 (6H, s, 2×$OCOCH_3$), 2.51 (1H, br.s, C_{18}-H), 3.67 (3H, s, $COOCH_3$), 4.54 (1H, dd, J=4.1 and 12.0Hz, C_3-H), 5.37 (1H, m, C_{12}-H), 5.51 (1H, d, J=7.0Hz, anomeric H of C_{28}-D-glucose)

Ilexoside H (11)

mp: 187-190° (amorphous); IR (cm^{-1}, KBr): 3400 (OH), 1735 (ester), 1100-1000 (glycoside); UV (λmax in MeOH): 248.0nm (ε=9800); ^{1}H-NMR ($CD_3OD/CDCl_3$=1:1): 0.85, 0.93, 1.00 (15H, 5×CH_3), 1.06 (3H, d, J=7.0Hz, C_{30}-H_3), 1.73 (3H, s, -C=C-CH_3), 5.35 (1H, m, C_{12}-H)

Ilexoside H acetate (11a)

mp: 148-150° (amorphous); IR (cm^{-1}, KBr): 1760 (ester); ^{1}H-NMR ($CDCl_3$): 0.78, 0.85, 0.94, 0.99 (18H, 6×CH_3), 1.68 (3H, s, -C=C-CH_3), 2.00 (7×3H, s, 7×$OCOCH_3$), 2.05 (2×3H, s, 2×$OCOCH_3$), 2.09 (3H, s, 1×$OCOCH_3$), 3.06 (1H, m, C_3-H), 4.49 (1H, d, J=6.2Hz, anomeric H of D-xylose), 4.69 (1H, d, J=7.2Hz, anomeric H of D-glucose), 5.64 (1H, d, J=7.3Hz, anomeric H of C_{28}-D-glucose), 5.34 (1H, m, C_{12}-H)

Alkaline hydrolysis of ilexosides A, E, H, K and O

Each saponin (100mg) was refluxed with 5% KOH in 50% EtOH solution for 4hrs under nitrogen. After cooling, the hydrolysate was acidified with d-H_2SO_4 to pH 3-4, freed from EtOH and ex-

tracted with BuOH. The BuOH extract was washed with water and concentrated to give a residue. Prosapogenin A (**6**), pubescenic acid (**9**), prosapogenin H (**12**), ilexosides D (**2**) and J (**3**) were obtained from ilexosides A (**1**), E (**8**), H (**11**), K (**4**) and O (**5**), respectively.

Alkaline treatment of ilexoside E monomethyl ester (**8b**) gave pubescenic acid monomethyl ester (**10**) by the above procedure.

Prosapogenin A (6)

mp: 257-259° (MeOH); $[\alpha]_D^{23}$: +0.352 (c = 0.20%, THF); IR (cm^{-1}, KBr): 3595, 3400 (OH), 1690 (COOH), 1100-1000 (glycoside); ^{1}H-NMR ($CD_3OD/CDCl_3$ = 1:1, TMS) δ ppm: 0.79, 0.85, 0.94, 1.05, 1.17 ,1.29 (6 × 3H, each s, 6 × CH_3), 1.00 (3H, d, J = 6.8Hz, C_{30}-H_3), 2.78 (1H, br.s, C_{18}-H), 3.10 (1H, m, C_3-H), 4.33 (1H, d, J = 6.7Hz, anomeric H of D-xylose), 5.31 (1H, m, C_{12}-H)

Prosapogenin A methylester (6b)

mp: 178-180°: IR (cm^{-1}, KBr): 3450 (OH), 1730 (ester); ^{1}H-NMR ($CDCl_3$, TMS) δ ppm: 0.68, 0.80, 0.91, 0.96, 1.17, 1.23 (6 × 3H, each s, 6 × CH_3), 0.99 (3H, d, J = 7.0Hz, C_{30}-H_3), 2.82 (1H, br.s, C_{18}-H), 3.61 (3H, s, $COOCH_3$), 5.31 (1H, m, C_{12}-H)

Prosapogenin H (12)

mp: 214-218°; UV (λmax in MeOH): 247.5nm; IR (cm^{-1}, KBr): 3400 (OH), 1700 (COOH), 1100-1000 (glycoside); ^{1}H-NMR (CD_3OD): 0.86, 0.94, 1.00, 1.07 (6 × CH_3), 1.72 (3H, s, -C = C-CH_3), 4.45 (1H, d, J = 6.0Hz, anomeric H of D-xylose), 4.66 (1H, d, J = 7.0Hz, anomeric H of D-glucose), 5.32 (1H, m, C_3-H); ^{13}C-NMR see Table IV.

Pubescenic acid monomethyl ester (10)

mp: 194-6° (MeOH); IR (cm^{-1}, KBr): 3565, 3500 (OH), 1695 (COOH); ^{1}H-NMR ($CDCl_3$): 0.74 (2 × 3H, s, C_{25}-and C_{26}-H_3), 0.92 (3H, d, J = 7.0Hz, C_{30}-H_3), 1.19 (3H, s, C_{29}-H_3), 1.24 (3H, s, C_{27}-H_3), 1.39 (3H, s, C_{23}-H_3), 3.12 (1H, dd, J = 4 and 10.7Hz, C_3-H), 2.52 (1H, br.s, C_{18}-H), 3.66 (3H, s, $COOCH_3$), 5.33 (1H, m, C_{12}-H); MS (rel. int. %): m/z 516 (M^+, 11.5), 498 (M^+-H_2O, 5.9), 470 (71.7), 398 (20.8), 264 (5.7), 252 (16.4), 246 (11.8), 236 (6.9), 219 (11.8), 218 (12.6), 201 (26.8), 165 (12.2), 146 (100)

Pubescenic acid dimethyl ester (9b)[15]

Ms (rel. int. %): m/z 530 (M^+, 14.6), 512 (M^+-H_2O, 8.9), 470 (69.4), 398 (15.1), 278 (1.9), 252 (14.8), 260 (5.9), 250 (5.6), 219 (11.0), 218 (8.2), 201 (33.0), 179 (100), 146 (43.7)

Sugar analysis of each glycoside

Each glycoside (10mg) in 4NHCl/dioxane/benzene (3:1:2, v/v, 4.8m*l*) was refluxed for 4hrs. The hydrolysate was diluted with water and extracted with EtOAc. The aquous layer was concentrated *in vacuo* to give a residue. It stood in a NaOH desiccator under vacuo for 2 days. TLC was performed with the solvent system of $CHCl_3$/MeOH/H_2O (70:30:4), and detected with 2,3,5-triphenyltetrazolium chloride. GLC of its TMS-ether was carried out under the following condition: SE-54 fused silica capillary column (0.2mm × 12m), column temp. 130-180°C (rate 1° C/min), injection temp. 250°, FID temp. 280°, helium $\bar{\mu}$ = 17.4cm/sec.

ACKNOWLEGEMENTS

This study was supported by a Basic Research Grant from the Korean Science and Engineering Foundation (KOSEF). The authors thank Dr. Yoko Naya, a vice-director of Suntory Institute for Bioorganic Research, Osaska, Japan for the measurement of SI mass of saponins.

LITERATURE CITED

1. *"Directory of Chinese Materia Medica"*, (zhong Yao Da Ci Dian or 中藥大辞典) ed. by Jiangsu New Medical College, Shanghai Scientific and Technological Publisher, Shanghai, p441 (1977).
2. Quin, W.-J., Chiao, C.-Y., Fan, T.-T., Chang, K.-S. and Pei, L.-C.: Studies on the active constituents in the leaves of Ilex pubescens Hook. et Arn. var. glabar Change. Part II. *Chung Ts'ao Yao* **11**, 97 (1980); *C.A.* **94**, 20292r (1981).
3. Peking Inst. Pharm. Ind.: Pharmacological studies of constituents from Ilex pubescens var. glabra leaf in coronary heart disease. *Chung Ts'ao Yao* **11**, 358 (1980); *C.A.* **94**, 114380v (1981).
4. Quin, W.-J., Jiao, Z.-Y., Fan, Z.-T., Ghen, B.-Q., Lin, X.-Y. and Yao, J.-X.: Studies on the chemical constituents of the leaves of Ilex pubescens Hook. et Arn. var. glaber CHang. III. Structural elucidation of glaberide I. *Yao Hsueh Tung Pao* **15**, 669 (1980); *C.A.* **95**, 12637n (1981).
5. Peking Inst. Pharm. Ind.: Chemical consti-

tuents of the leaves of Ilex pubescens var. glabra. *Yao Hsueh Tung Pao* **15**, 42 (1980); *C.A.* **95**, 138448v (1981).

6. Lin, X.-Y., Yao, J.-X. and Zhang, S.: Crystal and molecular structure of glaberide I. *Huaxue Xuebao* **40**, 469 (1982); *C.A.* **97**, 52551c (1982).
7. Zhang, X., Ke, M. and Ou, F.: Extraction and isolation of total flavonoid glycosides of Mao Don Quing (Ilex pubescens) bark. *Zhongcaoyao* **12**, 399 (1981); *C.A.* **96**,196519a (1982).
8. Xu, J. and Cheng, B.: Reaction between ammonium hydroxide-calcium chloride and flavones and its application. *Zhongcayao* **13**, 502 (1982); *C.A.* **98**, 157840v (1983).
9. Zeng, L.M., Su, J.Y., Liu, D.W. and Ma, F.E.: Studies on the chemical structure of ilexolide A. *Chem. Nat. Prod. Proc. Sino-Am. Symp.* p280 (1980) (pub. 1982), ed. by Wang, Y. Sci. Press (Beijing, Peop. Rep. China); *C.A.* **98**, 157840v (1983).
10. Zeng, L.M., Su, J.Y. and Zhang, S.: Chemical structure of ilexolide A. *Gaodeng Xuexiao Huaxue Xuebao* **5**, 503 (1984): *C.A.* **101**, 226825p (1984).
11. Han, Y. N., Yang H.O. and Han, B.H.: Studies on the anticoagulant component of Artemisiae herba. *Yakhak Hoeji* **28**, 69 (1984).
12. Kosela, S., Rasad, A., Achmad, S.A., Wicaksonon, W., Baik, S.K., Han, Y.N. and Han, B.H.: Effects of diterpene acids on malondialdehyde generation during thrombin induced aggregation of rat platelets. *Arch. Pharm. Res.* **9**, 189 (1986).
13. Han, Y.N., Yoon, H.W., Kim, S.H. and Han, B.H.: Effects of perilla oil intake on bleeding time, thromboxane formation and platelet fatty acid in rats. *Kor. J. Pharmacog.* **18**, 5 (1987).
14. Han, Y.N., Han, B.H., Baik, S.K. and Kim, T.H.: Antithrombotic activities of saponins from *Ilex pubescens*. *Arch. Pharm. Res.* **10**, 115 (1987).
15. Han, Y.N., Baik, S.K., Kim, T.H. and Han, B.H.: Triterpenoids of Ilex pubescens. *Arch. Pharm. Res.* **10**, 121 (1987).
16. Seo, S., Tomita, Y., Tori K. and Yoshimura, Y.: Determination of the absolute configuration of a secondary hydroxy group in a chiral secondary alcohol using glycosidation shifts in carbon-13 nuclear magnetic resonance spectroscopy. *J. Amer. Chem. Soc.* **100**, 3331 (1978).
17. Tori, K., Seo, S., Yoshimura, Y., Arita, H. and Tomita, Y.: Glycosidation shifts in carbon-13 NMR spectroscopy: Carbon-13 signal shifts from aglycone and glucose to glucoside. *Tetrahedron Letters* No.2, 179 (1977).
18. Kambara, H. and Hishida, S.: Secondary ion mass spectra of nonvolatile bioorganic compounds. *Anal. Chem.* **53**, 2340 (1981).