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ILEXOSIDES E, F, G, H AND I, NOVEL 18,19-SECO-URSANE GLYCOSIDES FROM FRUIT OF *ILEX CRENATA*[1]

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Summary : From the fresh fruits of *Ilex crenata* has been isolated five new saponins named ilexosides E, F, G, H and I, respectively. Their structures were established on the basis of spectral and chemical evidence. They exhibited anti-allergic activities.

In the course of our search for bioactive metabolites from *Ilex* spp. we have already found four new anti-allergic saponins named ilexoside A, B, C, and D[2] in the fresh fruits of *Ilex crenata* Thunb. cultivated widely in Japan as a garden plant. The present paper deals with the isolation and structure determination of the five novel saponins named ilexosides E(**1**), F(**2**), G(**3**), H(**4**) and I(**5**).

Hot water extract of the fresh fruits(10 kg) of *Ilex crenata* Thunb., followed by treatment with Amberlite XAD-2 column chromatography gave a saponin fraction(150g). Repeated separation of saponin fraction by reversed-phase(ODS) and ordinary-phase SiO_2 column chromatography furnished five new saponins, ilexoside E(**1**, 120mg), F(**2**, 120mg), G(**3**, 60mg), H(**4**, 160mg) and I (**5**, 320mg).

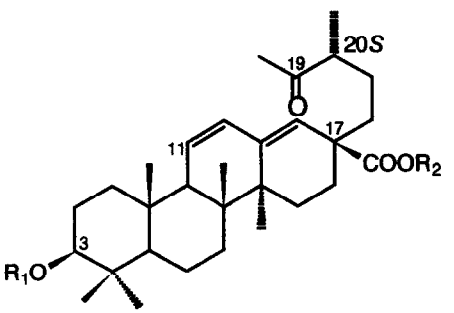
A cellulase treatment of **1** and **2** gave α-ilexanolic acid(**6**) for aglycone mp 234-236°C, $[\alpha]_D$ -38.7°(c=1.1, MeOH), UV(MeOH): λ_{max}243(ε18,700), $C_{30}H_{46}O_4$ showed a quasi-molecular ion peak at *m/z* 471$(M+H)^+$, *m/z* 493$(M+Na)^+$ in the positive FAB-MS.

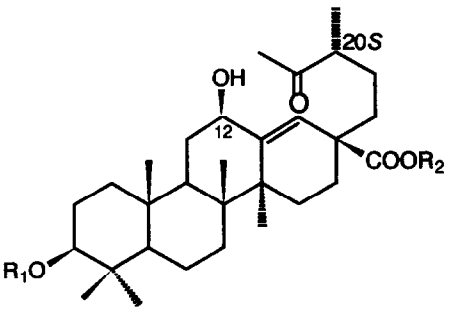
Ilexoside E(**1**), mp 171.5-172.5°C, $[\alpha]_D$ -67.0°(c=1.7, MeOH), has the molecular, $C_{52}H_{82}O_{21}$ $4H_2O$ based on the elementary analysis. On acid hydrolysis **1** afforded a diastereomeric mixture(**6'**)(20S:20R=1:1)[3] of **6** and L-arabinose, D-glucose, L-rhamnose and D-xylose in the ratio 1:1:1:1(HPLC). ^1H- and ^{13}C-NMR spectra of **1** indicated the presence of one α-arabinopyranosyl unit [H-1' δ 4.79(d, J=7.5Hz), C-1': δ 107.5], one β-glucopyranosyl unit[H-1':δ6.06(d, J=8.0Hz), C-1': δ 95.1], one α-rhamnopyranosyl unit[H-1': δ 6.39s, C-1': δ101.3] and one β-xylopyranosyl unit[H-1': δ4.82(d, J=7.5Hz), C-1': δ105.7]. A crude hesperidinase treatment of **1** gave ilexoside D(**7**)[2] and L-rhamnose.

Comparison of ^{13}C-NMR spectrum of **1** with that of **7** showed glycosylation shifts[4] for the C-2 signal(+5.6ppm) and C-3 signal(-4.1ppm) of glucosyl moiety, demonstrating that a α-rhamnopyranosyl group is located at the C-2-OH of glucose. Therefore, **1** was formulated as 3-O-α-L-arabinopyranosyl-α-ilexanolic acid 28-O-α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→6)]-β-D-glucopyranoside.

Ilexoside F(**2**), mp 183-185°C, $C_{58}H_{92}O_{26}$ $2H_2O$, $[\alpha]_D$ 37.2°(c=4.3, MeOH) showed similar spectral data(see Experimental) those of compound **1**. On acid hydrolysis, **2** furnished L-arabinose, D-glucose L-rhamnose and D-xylose in the ratio 1:2:1:1 besides **6'**. ^{1}H- and ^{13}C-NMR spectra of **2** indicated the presence of one α-arabinopyranosyl unit[H-1': δ 4.77 (d, J=7.5Hz), C-1': δ 107.5], two β-glucopyranosyl unit[H-1': δ 5.34(d,J=8.0Hz, C-1': δ 106.3, H-1' δ 6.07(d, J=7.0Hz), C-1': δ 95.0], one α-rhamnopyranosyl unit[H-1': δ 6.40s, C-1': δ 101.2] and one β-xylopyranosyl unit[H-1': δ 4.82(d, J=7.5Hz), C-1': δ 105.7]. A β-glucosidase treatment of **2** gave **1** and D-glucose. In the same way as **1**, a glycosylation shift was observed for the C-3 signal(+9.2ppm) of arabinosyl moiety, disclosing the site of glycosylation. Hence, **2** was formulated as 3-O-β-D-glucopyranosyl(1→3)-α-L-arabinopyranosyl-α-ilexanolic acid 28-O-α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl(1→6)]-β-D-glucopyranoside.

A cellulase treatment of **3-5** gave an aglycone, designated β-ilexanolic acid(**8**), amorph. powder, $[\alpha]_D$ -28.6°(c=1.9, MeOH), $C_{30}H_{48}O_5$, showed a quasi-





	R_1	R_2
1	-ara	-glc^6(xyl, rha^2)
2	-ara^{3}glc	-glc^6(xyl, rha^2)
6	-H	-H
6'	-H	-H (20*RS*)
7	-ara	-glc^6(xyl, rha^2)

	R_1	R_2
3	-ara	-H
4	-ara^{3}glc	-H
5	-ara^{3}glc	-glc
8	-H	-H
9	-H	-Me

Table 1 ^{13}C-NMR Data of compounds **1-8** (pyridine-d_5, 100MHz, δ-values)

	Aglycone Moeties								Saccharide Chains						
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**		**1**	**2**	**3**	**4**	**5**	**7**
C-1	38.3	38.4	39.8	39.7	39.5	38.5	38.4	39.7	C-1	107.5	107.5	107.6	107.3	107.4	107.2
2	26.7	26.7	26.9	26.9	26.9	27.8	26.6	28.6	2	72.9	72.0	72.9	71.9	71.9	73.0
3	89.0	88.8	88.8	88.7	88.8	77.9	88.8	78.1	3-Ara 3	75.0	84.2	74.6	84.1	84.0	74.6
4	39.9	39.9	39.8	39.8	39.8	39.5	39.8	39.6	4	69.2	69.2	69.3	69.2	69.3	69.3
5	55.7	55.6	56.2	56.2	56.2	55.3	55.7	56.2	5	66.7	67.1	66.8	66.9	67.0	66.5
6	18.6	18.5	18.5	18.5	18.5	18.6	18.4	18.8	C-1		106.3		106.2	106.1	
7	32.7	32.6	33.0	33.0	32.9	32.5	32.7	33.0	2		75.7		75.7	75.7	
8	41.4	41.3	41.3	41.4	41.5	40.8	41.2	41.5	3-Glc 3		78.7		78.6	78.6	
9	55.0	54.9	49.9	49.8	49.8	49.8	54.8	54.2	4		71.6		71.8	71.6	
10	37.0	36.9	37.4	37.4	37.4	37.0	36.9	37.8	5		78.4		78.4	78.3	
11	128.2	128.1	35.2	35.1	35.0	128.7	128.1	35.2	6		62.8		62.8	62.7	
12	130.6	131.5	69.5	69.2	69.3	130.4	130.4	69.2	C-1	95.1	95.0			96.3	96.2
13	143.8	143.6	145.2	146.4	147.7	142.7	143.7	146.5	2	79.6	79.6			79.3	74.0
14	41.9	41.8	43.8	43.4	43.9	41.5	41.7	43.9	28-Glc 3	74.6	74.8			71.2	78.7
15	26.7	26.7	28.8	28.6	28.8	26.6	26.6	28.3	4	70.7	70.9			78.7	71.3
16	28.2	28.0	29.7	29.5	29.4	27.4	28.2	29.5	5	77.8	77.8			62.5	77.8
17	48.3	48.2	47.6	47.1	47.5	47.5	47.7	47.1	6	69.5	69.4				69.3
18	126.6	126.5	122.5	122.6	121.0	127.7	126.9	122.5	C-1	105.7	105.8				105.5
19	213.1	212.3	212.4	212.0	212.3	211.8	211.6	212.0	2	74.8	74.9				74.6
20	47.5	47.4	47.8	47.6	47.7	47.4	47.9	47.6	28-Xyl 3	78.1	78.2				77.8
21	28.2	28.0	28.1	28.2	28.0	28.1	28.1	28.2	4	71.2	71.1				71.1
22	38.6	38.2	39.3	39.7	39.8	38.9	38.6	39.5	5	67.3	67.2				66.9
23	28.6	28.8	28.4	28.2	28.2	28.3	28.2	28.5	C-1	101.3	101.2				
24	17.0	16.6	17.0	16.8	16.5	16.0	16.4	16.5	2	72.6	72.2				
25	19.4	19.3	16.9	17.0	17.0	18.2	18.4	16.9	28-Rha 3	72.2	72.7				
26	17.0	17.1	18.5	18.3	18.3	18.2	17.0	18.4	4	73.9	73.9				
27	20.3	20.1	21.9	21.9	21.8	20.1	20.2	21.9	5	70.0	69.9				
28	175.5	175.5	178.3	178.4	175.5	177.9	174.9	178.1	6	18.6	18.5				
29	28.2	28.4	28.2	28.2	28.3	28.1	28.2	28.2							
30	16.7	16.9	16.5	16.4	16.5	16.4	16.4	16.6							

Table 2. ^{1}H-NMR Data of compounds **1-6** and **8** (pyridine-d_5/D_2O, 100MHz, δ-values, values in parenthesis are coupling constsnts in Hz).

H	1	2	3	4	5	6	8
3	3.34dd (11.0,4.0)	3.36dd (11.0,4.0)	3.36dd (11.0,4.0)	3.36dd (11.0,4.0)	3.35dd (11.0,4.0)	3.48dd (11.0,4.0)	3.46dd (11.0,4.0)
11	5.62d (10.0)	5.61d (10.5)				5.72d (10.5)	
12	6.23dd (10.0,2.0)	6.23dd (10.5,2.0)	4.66dd (10.5,2.0)	4.83dd (10.0,3.0)	4.62dd (9.5,6.0)	6.20dd (10.5,2.5)	4.67dd (9.5,6.0)
18	5.93s	5.93s	6.61s	6.62s	6.54s	5.87s	6.62s
23	1.24s	1.28s	1.28s	1.30s	1.29s	1.24s	1.24s
24	1.04s	0.79s	0.92s	0.92s	0.91s	0.94s	0.99s
25	0.86s	0.87s	1.00s	1.02s	1.06s	0.90s	1.05s
26	0.95s	0.89s	0.80s	0.77s	0.71s	0.89s	0.83s
27	1.04s	1.05s	1.25s	1.26s	1.22s	1.09s	1.24s
29	2.21s	2.21s	2.07s	2.09s	2.10s	2.15s	2.08s
30	1.14d (7.0)	1.15d (7.0)	0.98d (6.5)	1.01d (7.0)	1.00d (6.5)	1.07d (7.0)	1.00d (6.5)
Anomeric H	4.79d (7.5) 4.82d (7.5) 6.06d (8.0) 6.39s	4.77d (7.5) 4.82d (7.5) 5.34d (8.0) 6.07d (7.0) 6.40s	4.80d (7.5)	4.77d (7.5) 5.36d (8.0)	4.75d (7.5) 5.35d (7.5) 6.29d (7.5)		

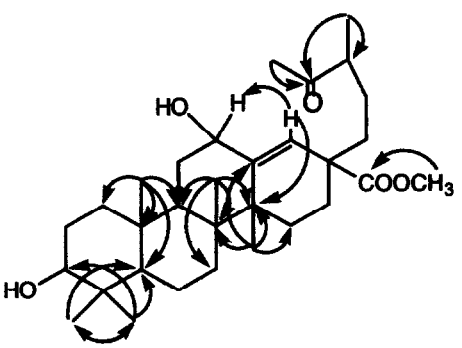


Fig 1 ^{1}H-^{13}C Long range couplings of **9**

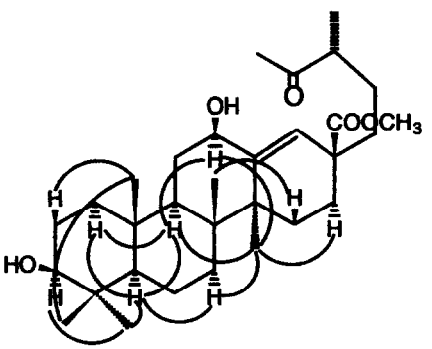


Fig 2 NOEs observed for **9**

molecular ion peak at m/z 511(M+Na)$^+$ in the positive FAB-MS. ^{13}C-NMR(INEPT spectrum) and ^{1}H-NMR(D_2O exchange) data indicated the presence of six methyls, nine methylenes, three methines, five quaternary carbons, one acetyl, two oxygen-bearing methines, one double bond, and one carboylic acid Methylation of **8** with CH_2N_2 afforded monomethylester(**9**), $C_{31}H_{50}O_5$[FAB-MS *m/z* 525(M+Na)$^+$]. ^{1}H-^{1}H COSY and ^{1}H homodecoupling experiments of **9** revealed for isolated spin systems(H-1~2, H-5~7, H-9~11~12, H-15~16, H-20~22, H-20~30). The gross structure of **9** was determined by analysis of NMR data including ^{1}H-^{1}H COSY, ^{13}C-^{1}H-COSY, COLOC and HMBC experiments(Fig.1). The β-orientaion of the C-12 hydroxy group was estimated from a large coupling(J=9.5, 6.0Hz) observed between H-11 and H-12 and NOE experiments(Fig.2). The remaining configuration at C-20 of **9** was determined to be *S* on the basis of the observation of positive cotton effect{$[\theta]_{287}$ + 370°}[5]. Accordingly, β-ilexanolic acid(**8**) was represented as 3β, 12β-dihydroxy-19-oxo-18, 19-seco-13(18)-urs-ene-28-oic acid.

Ilexoside G(**3**), mp 194-196°C, $[\alpha]_D$ -19.5°(c=0.8, MeOH), has the molecular, $C_{35}H_{56}O_9$ H_2O based on the elementary analysis. On acid hydrolysis,**3** afforded L-arabinose and **6'**. ^{1}H- and ^{13}C-NMR spectra of **3** indicated the presence of one α-arabinopyranosyl unit[H-1': δ4.80(d,J=7.5Hz), C-1': δ107.6]. Comparison of ^{13}C-NMR spectrum of **3** with that of **8** showed a glycosylation shift for the C-3 signal(+10.7ppm), demonstrating that a arabinopyranosyl group is located at the C-3-OH. Therefore, **3** was formulated as 3-O-α-L-arabinopyranosyl-β-ilexanolic acid.

The positive FAB-MS of ilexoside H(**4**), mp 217-219°C, $C_{41}H_{66}O_{14}$ $3/2H_2O$, $[\alpha]_D$ -11.2°(c=1.9, MeOH), showed similar spectral data(see Experimental) those of compound **3**. On acid hydrolysis, **4** furnished L-arabinose, D-glucose in the ratio 1:1 besides **6'**. ^{1}H- and ^{13}C-NMR spectra of **4** indicated the presence of one α-arabinopyranosyl unit[H-1': δ 4.77(d,J=7.5Hz),C-1':δ107.3] one β-glucopyranosyl unit[H-1': δ 5.36(d, J=8.0Hz, C-1': δ 106.2]. A β-glucosidase treatment of **4** gave **3** and D-glucose. In the same way as **3**, a glycosylation shift was observed for the C-3 signal(+9.5ppm) of arabinosyl moiety, disclosing the site of glycosylation. Hence, **4** was formulated as 3-O-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl-β-ilexanolic acid

Ilexoside I(**5**), mp 186-188°C, $C_{47}H_{76}O_{19}$ $2H_2O$, $[\alpha]_D$ -19.4°(c=2.4, MeOH), showed similar spectral data(see Experimental) those of compound **4**. On acid hydrolysis, **5** furnished L-arabinose and D-glucose in the ratio(1:2) besides **6'**. The ^{1}H- and ^{13}C-NMR spectra indicated the presence of one α-arabinopyranosyl unit[H-1': δ 4.75(d, J=7.5Hz), C-1': δ 107.4] and two β-glucopyranosyl unit[H-1': δ 5.35(d, J=7.5Hz), C-1': δ 106.1 H-1': δ 6.29(d, J= 7.5 Hz), C-1': δ 96.3]. Comparison of ^{13}C-NMR spectrum of **5** with those of **4** showed a glycosylation shift for the C-28 signal(-2.9ppm), indicating the presence of a glucosidic ester linkage of β-ilexanolic acid. Therefore, **5** was formulated as 3-O-β-D-glucopyranosyl(1→3)-α-L-arabinopyranosyl-β-ilexanolic acid

28-O-β-D-glucopyranoside. The pD_2 values of inhibitory effect of ilexosides E, F, G, H and I on histamine release from must cells induced by concanavalin A^6 were 5.83, 5.75, 5.8, 5.6 and 5.57, respectively, which were about 10 times as potent as glycyrrhizin(pD_2=4.7).

Expermental Section

General Methods. Melting points were measured with a Yanagimoto micromelting point apparatus and were uncorrected. Optical rotations were taken on a JASCO J-20A digital polarimeter. IR spectra were taken on a Hitachi IR-27G. NMR spectra were recorded on a JEOL GX-400 spectrometer in C_5D_5N solution using TMS as an internal standard. NMR experiments included ^1H-^1H-COSY, ^{13}C-^1H-COSY, INEPT, proton decouplings, difference NOE, COLOC and HMBC (512 x 1024 data matrix size, 128 scans, recycle delay=1.16s). Coupling constants(J values) are given in hertz(Hz). The EI- and FAB-MS(Xe gun, 10kV, *m*-nitrobenzyl alcohol as matrix) were measured on Shimadzu LKB-9000B and JEOL JMS-PX303 mass spectrometer, respectively. For column chromatography, Kiesel gel 60(230-400 mesh, Merck) was used. TLC was carried out on silica gel 60F-254(Merck) with the following system: CH_3Cl-MeOH-H_2O(25:2:0.1).

Isolation of saponins. The fresh fruits(10kg) of *Ilex crenata* were extracted with hot H_2O(3hr). The water extract was passed through an Amberlite XAD-2 column and eluated with MeOH. Crude saponin(150g) obtained by evaporation of the MeOH eluate were treated with Servachrome XAD-2(40-70% MeOH) to give four fractions, fr.1~6 in order to elution. Fr.2 was repeatedly chromatographed on silica gel with CH_3Cl-MeOH-H_2O(25:4:0.1), CH_3Cl-MeOH-AcOEt-H_2O(2:2:4:1, lower phase) and ODS(40~60%MeOH) to afford ilexosides A (90mg), B(3.7g) and H(**4**,160mg) and I(**5**,320mg). Fr.3 by silica gel column chromatography, eluating with CH_3Cl-MeOH-H_2O(25:8:0.1) and then with CH_3Cl-MeOH-AcOEt-H_2O(2:2:4:1, lower phase), gave ilexoside C(300mg), D(**7**, 220mg) and G(**3**, 60mg). Fr.5 was subjected to a silica gel column with CH_3Cl-MeOH-H_2O(65:35:10,lower phase) and n-BuOH-AcOEt-H_2O(4:1:5, upper phase) to afford ilexosides E(**1**, 120mg) and F(**2**, 120mg).

Ilexoside E(1). Mp 171.5-172.5°C, $[\alpha]_D$ -67.0°(c=1.7, MeOH).(Found: C,55.99;H,8.02. $C_{52}H_{82}O_{21}$ $4H_2O$ requires: C56.00;H,8.41). IR(KBr): ν_{max}3400(br, OH), 1740(ester C=O), 1700(acetyl C=O), 1640(C=C), 1060, 1030. UV(MeOH): ν_{max}235(33,800), 242(36,300), 252sh, 280(80).CD(MeOH) $[\theta]_{280}$ +70, $[\theta]_{242}$ -6.15x10^4. FAB-MS *m/z* 1065$(M+Na)^+$. EI-MS *m/z* 470(genin)$^+$, 452(genin-H_2O)$^+$, 426(genin-CO_2)$^+$, 408, 371, 353, 99. For NMR data, see Tables 1 and 2.

Ilexoside F(2). Mp 183-185°C, $[\alpha]_D$ -37.2°(c=4.3, MeOH).(Found:C,56.00 H,7.65. $C_{58}H_{92}O_{26}$ $2H_2O$ requires: C56.12;H,7.79).IR(KBr): ν_{max}3450(br, OH), 1745(ester C=O), 1705(acetyl C=O), 1640(C=C), 1070, 1030. UV(MeOH):ν_{max}235 (22,000), 242(24,000), 252sh, 281(130). CD(MeOH) $[\theta]_{280}$ +365, $[\theta]_{242}$ 5.30x10^4

FAB-MS *m/z* 1227 $(M+Na)^+$. EI-MS *m/z* 470(genin)$^+$, 452(genin-H_2O)$^+$, 426(genin-CO_2)$^+$, 408, 371, 353, 99. For NMR data, see Tables 1 and 2.

Ilexoside G(3). Mp 194-196°C, $[\alpha]_D$ -19.4°(c=0.8, MeOH).(Found:C,66.64 H,8.99. $C_{35}H_{56}O_9$ H_2O requires: C66.38;H,9.26). IR(KBr): ν_{max}3400(br, OH), 1700(acetyl C=O), 1690(C=O), 1640(C=C), 1070, 1050. UV(MeOH): ν_{max}280(140). FAB-MS *m/z* 643 $(M+Na)^+$. EI-MS *m/z* 470(genin)+, 452(genin-H_2O)+, 426(genin-CO_2)$^+$, 408, 371, 353, 99. For NMR data, see Tables 1 and 2.

Ilexoside H(4). Mp 217-219C°, $[\alpha]_D$ -11.2°(c=1.9, MeOH).(Found:C,60.96 H,8.72. $C_{41}H_{66}O_{14}$ 3/2H_2O requires: C60.80;H,8.72).IR(KBr): ν_{max}3400(br, OH), 1705(acetyl C=O), 1695(C=O), 1640(C=C), 1075, 1025. UV(MeOH): ν_{max}277(170). CD(MeOH) $[\theta]_{325}$ +270. FAB-MS *m/z* 805 $(M+Na)^+$. EI-MS *m/z* 488 (genin)$^+$, 470((genin-H_2O)$^+$, 389, 353, 99. For NMR data, see Tables 1 and 2.

Ilexoside I(5). Mp 186-188°C, $[\alpha]_D$ -19.4°(c=2.4, MeOH).(Found:C,57.24 H,8.41. $C_{47}H_{76}O_{19}$ 2H_2O requires: C57.53;H,8.22).IR(KBr): ν_{max}3450(br, OH), 1740(ester C=O), 1700(acetyl C=O), 1640(C=C), 1075, 1020. UV(MeOH)ν_{max}279: (110). CD(MeOH) $[\theta]_{305}$ +85. FAB-MS *m/z* 967 $(M+Na)^+$. EI-MS *m/z* 488(genin)$^+$, 470 (genin-H_2O)$^+$, 389, 353, 99. For NMR data, see Tables 1 and 2.

Enzymatic hydrolysis of ilexosides E(1) and F(2). Ilexoside E(1) (35mg) or F(2)(40mg) was taken in EtOH-H_2O(1:9) and 0.01M NaH_2PO_4 buffer(pH 4.0) 5ml each, incubated with crude cellulase (50mg, Siguma) for two weeks at 37°C and work-up as usual. The crude genin was chromtographed on a silica gel column with CH_3Cl-MeOH-H_2O(25:4:0.1) giving α-ilexanolic acid(6, 12mg), mp 234-236°C, $[\alpha]_D$ -38.7°(c=1.0, MeOH).(Found:C,76.49;H,10.01. $C_{30}H_{46}O_4$ requires: C76.55;H,9.85). IR (KBr): ν_{max}3450(br, OH),1700(acetyl C=O), 1695, 1640(C=C), 1075, 1030. UV(MeOH): ν_{max}280(150), 243(18,700). CD(MeOH) $[\theta]_{290}$ +450, $[\theta]_{250}$ -5.13x10^4, $[\theta]_{235}$ -3.99 x10^4. FAB-MS *m/z* 493 $(M+Na)^+$. EI-MS *m/z* 470(M^+), 426(M^+-CO_2), 371, 353, 99. For NMR data, see Tables 1 and 2.

Enzymatic hydrolysis of ilexosides E(1). Ilexoside E(1)(35mg) was taken in EtOH-H_2O(1:9) and 0.01M NaH_2PO_4 buffer(pH 4.0) 2ml each, incubated with crude hesperidinase (30mg, Tanabe) for 4hr at 37°C and work-up as usual. The crude sapogenin was chromtographed on a silica gel column with CH_3Cl-MeOH-H_2O(25:8:0.5) giving ilexoside D(7, 10mg),

Acid hydrolysis of ilexoside E(1). A solution of 1(20mg) in 5% H_2SO_4 in 50 % EtOH was heated at 100°C for 3hr. The usual work-up gave diastereomeric mixture(6', 8mg). Compound 6': mp 211-212°, $[\alpha]_D$ -46.0°(c=0.6, MeOH). IR(KBr): ν_{max}3450(br, OH), 1700(acetyl C=O), 1695, 1640(C=C),1075, 1020. UV(MeOH): ν_{max}280(150),243(17,000). CD(MeOH) $[\theta]_{253}$ -4.5x10^4, $[\theta]_{234}$ -5.2x10^4. FAB-MS *m/z* 493 $(M+Na)^+$. EI-MS *m/z* 470(M^+), 426(M^+-CO_2), 371, 353, 99. For NMR data, see Tables 1 and 2. The aqueous layer was neutralized with Amberlite IR-45 and evaporated in vacuo to dryness. The mole ratio and D(L) of each suger was determined by using RI detection(waters 410) and chiral detection (Shodex OR-1), respectively in HPLC(Shodex RSpak DC-613, 75%CH_3CN, 1ml/min, 70°C) by comparison with authentic sugars(10mM each of L-ara, L-

rha, D-glc and D-xyl). These sugars gave peaks as follows : L(+)-rha; 4.8min D(+)-xyl; 5.75 min, L(+)-ara; 6.2min, D(+)-glc; 7.38min.

Acid hydrolysis of ilexosides F(2). Acid hydrolysis of **2**(25mg) was carried out in the same way as for **1** to give **6'**(10mg, TLC, mp, IR, EI-MS) well as L-rha, D-xyl, L-ara, D-glc.

Enzymatic hydrolysis of ilexosides G(3), H(4) and I(5). Enzymatic hydrolysis of ilexoside G(**3**)(25mg) or H(**4**)(32mg) or I(**5**)(38mg) was carried out in the same way as for **1** to give the β-ilexanolic acid(**8**, 15mg), powder, $[\alpha]_D$-28.6°(c=1.9, MeOH).(Found:C,73.50;H,10.00. $C_{30}H_{48}O_5$. requires: C73.73;H,9.90). IR(KBr): ν_{max}3400(br, OH), 1705(acetyl C=O), 1695 (C=O), 1640 (C=C), 1075, 1030. UV(MeOH) : ν_{max}281(180), CD(MeOH)$[\theta]_{318}$ +80. FAB-MS *m/z* 511(M+Na)$^+$. EI-MS *m/z* 488(M^+), 470(M^+-H_2O), 426(M^+-CO_2-H_2O), 389, 353, 99. For NMR data, see Tables 1 and 2.

Acid hydrolysis of ilexoside G(3). Acid hydrolysis of **3**(10mg) was carried out in the same way as for **1** to give **6'**(4mg, TLC, mp, IR, EI-MS) as well as L-ara.

Acid hydrolysis of ilexoside H(4). Acid hydrolysis of **4**(13mg) was carried out in the same way as for **1** to give **6'**(4mg, TLC, mp, IR, EI-MS) as well as L-ara, D-glc.

Acid hydrolysis of ilexoside I(5). Acid hydrolysis of **5**(15mg) was carried out in the same way as for **1** to give **6'**(3.5mg, TLC, mp, IR, EI-MS) as well as L-ara, D-glc.

Methylation of β-ilexanolic acid(8). Compound **8**(20mg) in ether was treated CH_2N_2-Et_2O to give monomethylester(**9**, 20mg). Compound **9**: amorph. $[\alpha]_D$ -27.5°(c=0.8, MeOH). UV(MeOH): ν_{max}279(150). CD(MeOH)$[\theta]_{287}$ +370. FAB-MS *m/z* 525(M+Na)$^+$. EI-MS *m/z* 502(M^+), 484(M^+-H_2O), 442(M^+-HCOOMe), 424(M^+-HCOOMe-H_2O), 99. ^{1}H-NMR($CDCl_3$): δ 0.76(3H,s), 0.80(3H,s), 0.87(3H,s), 0.98 (3H,s), 1.07(3H,d,J=7.0Hz), 1.09(3H,s), 2.11(3H,s), 3.22(1H,dd,J=11.0,5.0Hz) 3.65 (3H,s), 4.22(1H,dd,J=9.5,6.0Hz), 5.67(1H,s).

References and note

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