Bull. Pharm. Sci., Assiut University, Vol. 21, Part 1, June 1998, pp. 27-36.

TRITERPENOIDAL SAPONINS FROM *KOCHIA INDICA* WIGHT.

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تم فصل سبعة مركبات صابونينية لأول مرة من جنس الكوشيا وذلك من نبات الكوشيا انديكا ويت الذى ينمو فى مصر.

وتحتوى كل هذه المركبات على حامض الجلوكيورنيك كأحد السكاكر الموجودة فى هذه المركبات متصلا بذرة الكربون رقم ٣ فى حامض الأوليانوليك وقد وجد أن المركبات من (٤-٧) هى مركبات سيكوجليوكوزيدية.

وقد تم التعرف الكامل على هذه المركبات بواسطة الطرق المختلفة للتحليل الكيميائى والطيفى وكذلك مقارنة النتائج بنظيرتها المنشورة سابقا.

Seven oleanolic acid saponins (1-7) were isolated from the aerial parts of Kochia indica Wight. (Chenopodiaceae), of which saponins (4-7) are seco-glycosides containing acidic substituents at C-3 of glucuronic acid. Compounds (1-6) have been isolated for the first time from genus Kochia and compound (7) is first reported from family Chenopodiaceae. The structures of the isolated compounds were characterized by different spectroscopic methods and by comparison with previously reported data.

INTRODUCTION

The genus *Kochia* (Chenopodiaceae) is represented by 35 species occurring as herbs or undershrubs distributed in temperate regions, Australia, South Africa and India.[1-3] The genus is represented in Egypt by one wild species viz. *K. indica*[1,2] and another ornamental plant viz. *K. scoparia*.[4]

In India, the plant is used in folk medicine as cardiac stimulant.[3] Also, *K. scoparia* which is native to Poland is used for the same purpose and as anti-rheumatic.[5]

Except for the isolation of harmane and harmine alkaloids from aerial parts of *K. scoparia*,[5] nothing could be traced in literatures concerning the chemical components of this genus. So, it seems interesting to investigate more deeply the constituents of this genus.

This work describes the isolation and structural characterization of seven triterpenoidal saponins from aerial parts of *Kochia indica* using different chromatographic techniques and various tools of spectral analyses; four of them (**4-7**) are seco-glycosides. Moreover, compounds (**1-6**) are described for the first time from genus *Kochia*, while compound (**7**) is first reported from family Chenopodiaceae.

EXPERIMENTAL

Nuclear Magnetic Resonance (400 MHz for ^{1}H and 100 MHz for ^{13}C) spectra were recorded in C_5D_5N on a JEOL JNM α-400 spectrometer using TMS as internal standard. Mass spectra were taken on a JEOL JMS-SX 102 spectrometer by direct inlet method at an ionizing voltage of 70 eV. For column chromatography, Diaion HP 20 (Mitsubishi), Kieselgel 60 (70-230 mesh, Merck) and LiChroprep. RP-18 (Merck) were used. For thin layer chromatography, silica gel 60 precoated

Received in 28/12/1997 & Accepted in 19/2/1998

Note: There was a recent work describing three new triterpenoidal saponins from fruits of *K. scoparia* by M. Yoshikawa *et al.*, Chem. Pharm. Bull. 45 (8), 1300 (1997).

plates, F-254 (Merck) were used. HPTLC was carried out using RP-18 precoated plates F-254s (Merck).

Plant material

The aerial parts of *K. indica* were collected from Assiut valley, Assiut, Egypt in May 1995. The plant was identified by Prof. A. Fayed, Dept. of Botany, Faculty of Science, Assiut University. A voucher specimen is deposited at the Herbarium of Dept. of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt.

Extraction and isolation

The air-dried powdered aerial parts of *K. indica* (3 kg) were extracted with methanol. The solvent free residue (220 g) was partitioned between ethyl acetate and water. The aqueous fraction (170 g) was applied to a column of Diaion HP 20 (150x5 cm; 1 kg of stationary phase) and eluted with H_2O, 50% MeOH, 80% MeOH and finally with MeOH (fraction volume 3L each). The 80% methanolic eluate (6.4 g) was chromatographed on a silica gel CC using $CHCl_3$-MeOH-H_2O (30:10:1) to (6:4:1) gradient to give four main fractions. Fraction no. 2 (524 mg) was subjected to a column of reversed phase RP-18 using 80% aq. MeOH to afford compound **1** (99 mg). Fraction no. 3 (844 mg) was chromatographed on a column of reversed phase RP-18 using 60% aq. MeOH to yield compound **2** (116 mg). Fraction no. 4 (770 mg) was methylated using ethereal diazomethane, then subjected to repeated and extensive column chromatography using reversed phase RP-18 and 80% aq. MeOH as eluent to obtain compounds **3** (8 mg), **4** (62 mg), **5** (10 mg), **6** (39 mg) and **7** (24 mg).

Compound (1): Chikusetsusaponin IVa, amorphous powder; R_f= 0.57, $CHCl_3$-MeOH-H_2O (6:4:1). FAB-MS (negative) m/z: 793 $[M-H]^-$ $C_{42}H_{65}O_{14}$. ^{1}H-NMR (C_5D_5N): δ 0.80, 0.85, 0.88, 0.96, 1.05, 1.25, 1.27 (each 3H, *s*, -Me x 7), 3.15 (1H, *dd*, *J*= 4.1, 13.4 Hz, H-18), 3.35 (1H, *dd*, *J*= 4.3, 11.6 Hz, H-3), 4.99 (1H, *d*, *J*= 7.8 Hz, glcA H-1), 5.38 (1H, *br.s.*, H-12) and 6.28 (1H, *d*, *J*= 8.0 Hz, glc H-1). ^{13}C-NMR (C_5D_5N, Tables 1 and 2).

Compound (2): Chikusetsusaponin V, amorphous powder; R_f= 0.42, $CHCl_3$-MeOH-H_2O (6:4:1). FAB-MS (negative) m/z: 955 $[M-H]^-$ $C_{48}H_{75}O_{19}$. ^{1}H-NMR (C_5D_5N): δ 0.80, 0.85, 0.88, 1.05, 1.07, 1.23, 1.24 (each 3H, *s*, -Me x 7), 3.15 (1H, *dd*, *J*= 4.1, 13.7 Hz, H-18), 3.24 (1H, *dd*, *J*= 3.9, 11.7 Hz, H-3), 4.97 (1H, *d*, *J*= 7.3 Hz, glcA H-1), 5.36 (1H, *br.s.*, H-12), 5.38 (1H, *d*, *J*= 7.8 Hz, glc H-1) and 6.28 (1H, *d*, *J*= 8.0 Hz, glc H-1). ^{13}C-NMR (C_5D_5N, Tables 1 and 2).

Compound (3): Momordin IIa methyl ester, amorphous powder; R_f= 0.28, $CHCl_3$-MeOH (8.5:1.5). FAB-MS (negative) m/z: 939 $[M-H]^-$ $C_{48}H_{75}O_{18}$. ^{1}H-NMR (C_5D_5N): δ 0.75, 0.77, 0.85, 1.16, 1.23, 1.34, 1.36 (each 3H, *s*, -Me x 7), 3.16 (1H, *m*, H-18), 3.31 (1H, *dd*, *J*= 4.1, 11.9 Hz, H-3), 3.59 (3H, *s*, -OMe), glcA H-1 obscured with H_2O signal, 5.31 (1H, *d*, *J*= 7.0 Hz, ara H-1), 5.40 (1H, *br.s.*, H-12) and 6.31 (1H, *d*, *J*= 8.2 Hz, glc H-1). ^{13}C-NMR (C_5D_5N, Tables 1 and 2).

Compound (4): Betavulgaroside III methyl ester, amorphous powder; R_f= 0.35, $CHCl_3$-MeOH (8.5:1.5). FAB-MS (negative) m/z: 997 $[M-H]^-$ $C_{50}H_{77}O_{20}$. ^{1}H-NMR (C_5D_5N): δ 0.78, 0.85, 0.88, 0.89, 1.04, 1.20, 1.23 (each 3H, *s*, -Me x 7), 3.15 (1H, *dd*, *J*= 4.1, 13.4 Hz, H-18), 3.27 (1H, *dd*, *J*= 4.4, 11.5 Hz, H-3), 3.47, 3.65, 3.67 (each 3H, *s*, -OMe x 3), 4.70 (1H, *d*, *J*= 16.6 Hz, H-2″a), 4.87 (1H, *d*, *J*= 7.8 Hz, glcA H-1), 5.11 (1H, *d*, *J*= 3.2 Hz, H-2′), 5.14 (1H, *d*, *J*= 16.6 Hz, H-2″b), 5.38 (1H, *br.s.*, H-12), 6.0 (1H, *d*, *J*= 3.2 Hz, H-3′) and 6.28 (1H, *d*, *J*= 8.1 Hz, glc H-1). ^{13}C-NMR (C_5D_5N, Tables 1 and 2).

Compound (5): Betavulgaroside IV methyl ester, amorphous powder; R_f= 0.47, $CHCl_3$-MeOH (8.5:1.5). FAB-MS (negative) m/z: 849 $[M-H]^-$ $C_{45}H_{69}O_{15}$. ^{1}H-NMR (C_5D_5N): δ 0.78, 0.80, 0.90, 0.91, 0.92, 1.21, 1.23 (each 3H, *s*, -Me x 7), 3.07 (1H, *dd*, *J*= 3.9, 13.4 Hz, H-

18), 3.35 (1H, *dd*, *J*= 4.1, 11.7 Hz, H-3), 3.48, 3.66 (each 3H, *s*, -OMe x 2), 3.68 (6H, *s*, -OMe x 2), 4.72 (1H, *d*, *J*= 16.6 Hz, H-2″a), 4.90 (1H, *d*, *J*= 8.0 Hz, glcA H-1), 5.14 (1H, *d*, *J*= 3.2 Hz, H-2′), 5.21 (1H, *d*, *J*= 16.6 Hz, H-2″b), 5.34 (1H, *br.s.*, H-12) and 6.03 (1H, *d*, *J*= 3.2 Hz, H-3′). ^{13}C-NMR (C_5D_5N, Tables 1 and 2).

Compound (6): Betavulgaroside V methyl ester, amorphous powder; R_f= 0.26, $CHCl_3$-MeOH (8.5:1.5). FAB-MS (negative) m/z: 1159 $[M-H]^-$ $C_{56}H_{87}O_{25}$. ^{1}H-NMR (C_5D_5N): δ 0.73, 0.80, 0.82, 0.93, 0.97, 1.11, 1.15 (each 3H, *s*, -Me x 7), 3.07 (1H, *m*, H-18), 3.17 (1H, *dd*, *J*= 4.2, 11.5 Hz, H-3), 3.38, 3.57, 3.65 (each 3H, *s*, -OMe x 3), 4.54 (1H, *d*, *J*= 16.4 Hz, H-2″a), 4.90 (1H, *d*, *J*= 7.0 Hz, glcA H-1), 5.11 (1H, *d*, *J*= 2.5 Hz, H-2′), 5.32 (1H, *br.s.*, H-12), 5.46 (1H, *d*, *J*= 7.6 Hz, glc H-1), 5.87 (1H, *d*, *J*= 16.4 Hz, H-2″b), 5.97 (1H, *d*, *J*= 2.5 Hz, H-3′) and 6.16 (1H, *d*, *J*= 8.1 Hz, glc H-1). ^{13}C-NMR (C_5D_5N, Tables 1 and 2).

Compound (7): 3-*O*-{2′-(2″-*O*-glycolyl)-glyoxylyl-ß-D-glucuronopyranosyl} 28-*O*-ß-D-glucopyranosyl oleanolic acid methyl ester, amorphous powder; R_f= 0.38, $CHCl_3$-MeOH (8.5:1.5). FAB-MS (negative) m/z: 967 $[M-H]^-$ $C_{49}H_{75}O_{19}$. ^{1}H-NMR (C_5D_5N): δ 0.78, 0.85, 0.87, 0.89, 1.06, 1.21, 1.24 (each 3H, *s*, -Me x 7), 3.16 (1H, *dd*, *J*= 4.1, 13.9 Hz, H-18), 3.27 (1H, *dd*, *J*= 4.4, 11.9 Hz, H-3), 3.53, 3.55, 3.68 (each 3H, *s*, -OMe x 3), 4.85 (1H, *d*, *J*= 7.8 Hz, glcA H-1), 4.90 (1H, *d*, *J*= 16.3 Hz, H-2″a), 5.06 (1H, *d*, *J*= 16.3 Hz, H-2″b), 5.39 (1H, *br.s.*, H-12), 6.24 (1H, *s*, H-2′) and 6.30 (1H, *d*, *J*= 8.1, glc H-1). ^{13}C-NMR (C_5D_5N, Tables 1 and 2).

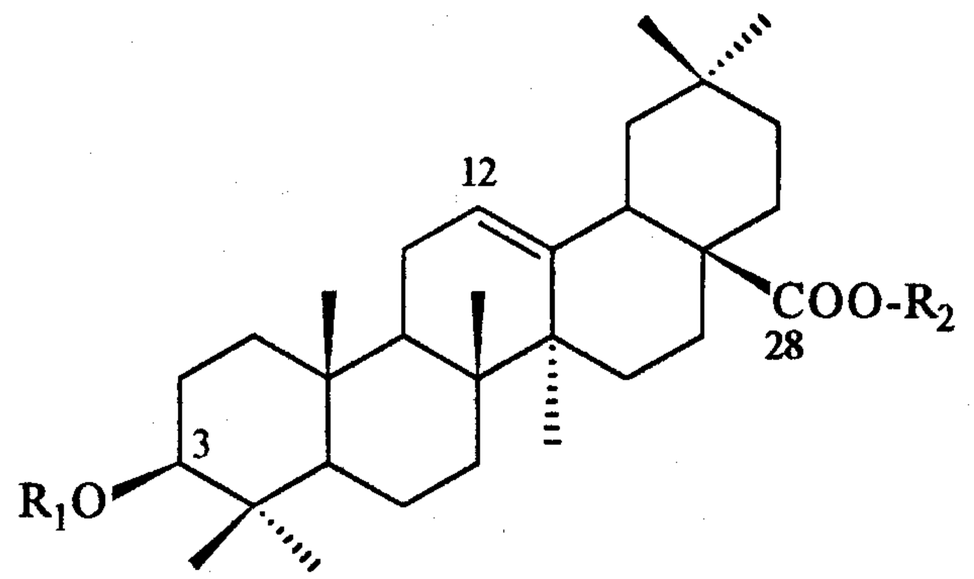
Acid hydrolysis of compounds (1-7): About 5 mg of each compound were dissolved in 10% HCl in dioxan-H_2O (1:1) (15 ml) and refluxed at 80°C for 3 hrs. The reaction mixture in each case was diluted with H_2O and then extracted with ether. From the ethereal layer, the aglycone was isolated and identified as oleanolic acid in case of saponins (**1-4**), (**6**) and (**7**) by direct comparison with authentic sample, while in case of saponin (**5**) the aglycone was identified as methyl oleanolate prepared by diazomethane methylation of oleanolic acid. In the aqueous phase, the normal sugar part of each saponin was identified by comparison with authentic substances using TLC precoated silica gel plates developed with acetonitrile-water (8.5:1.5) as a solvent system (triple run). Other unusual seco-compounds were identified from their spectral data.

Alkaline hydrolysis of compound (1): A solution of **1** (10 mg) in 5% aq. KOH (5 ml) was heated under reflux for 4 hrs. The reaction mixture was neutralized and passed over Diaion HP 20 column chromatography and eluted with H_2O to afford the sugar part which was identified as D-glucose comparing with authentic material.

RESULTS AND DISCUSSION

The aerial parts of *K. indica* were extracted with MeOH, and the extract was then partitioned with EtOAc and H_2O. The aqueous fraction was applied on a column of Diaion HP 20 and eluted with H_2O, 50% MeOH, 80% MeOH and finally with MeOH. From the crude saponin fraction eluted with 80% MeOH, two acidic saponins (**1** and **2**) were isolated as free acids and five compounds (**3-7**) have been obtained as their methyl esters after methylation using ethereal diazomethane.

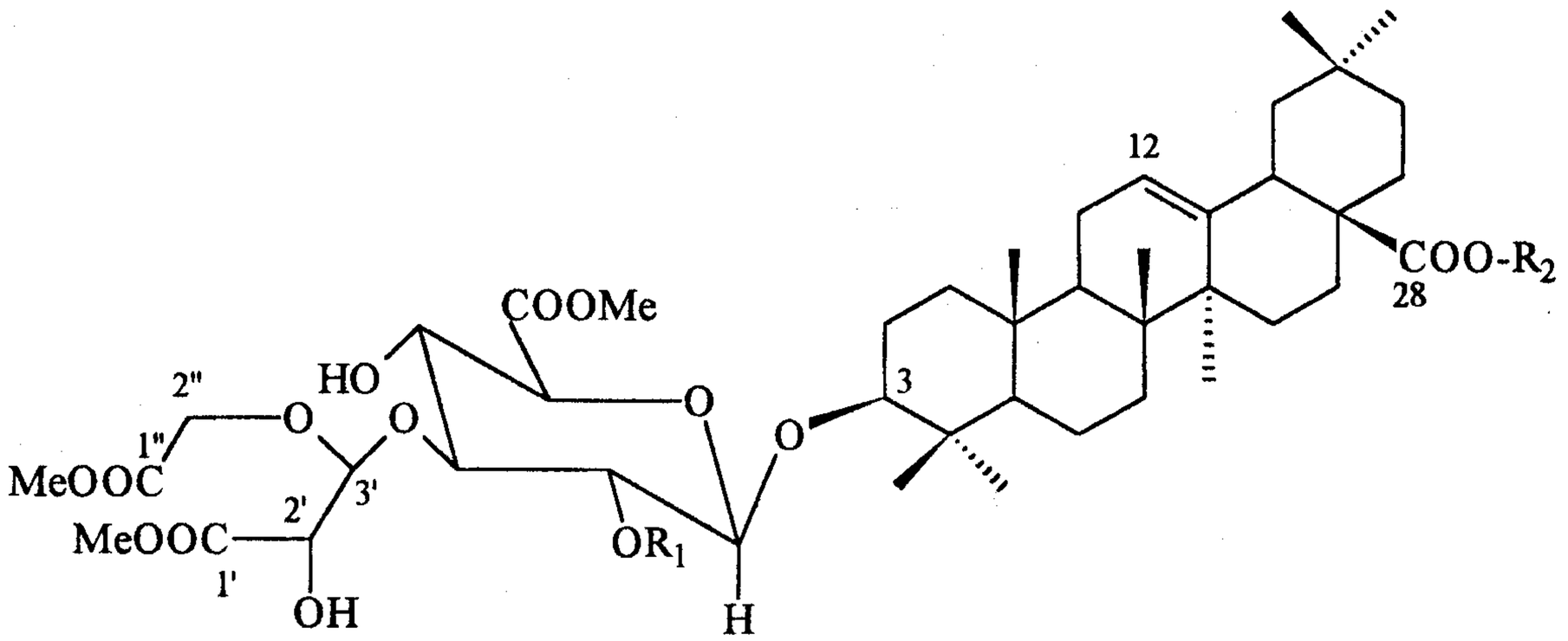
The ^{1}H-NMR of saponin (**1**) showed seven sharp tertiary methyl signals at δ_H 0.80, 0.85, 0.88, 0.96, 1.05, 1.25 and 1.27 (each 3H, *s*, -Me x 7). These results together with ^{13}C-NMR spectral data at δc 122.9, 144.1 and 176.4 and other carbon signals of (**1**) (Table 1) are indicative of an $\Delta^{12\text{-}13}$ oleanene skeleton having a carboxyl function at C-28 i.e. oleanolate saponin.[6] This was confirmed by acid hydrolysis which afforded besides D-glucuronic acid and D-glucose an aglycone which was identified as oleanolic acid by direct authentication (mmp. and cochromatography). The ^{1}H-NMR of (**1**) which displayed a downfield ß-anomeric proton of a ß-D-glucose at δ_H 6.28, 1H, *d*, *J*= 8.0 Hz suggesting an ester linked sugar.[6,7] This was confirmed by the upfield ^{13}C-NMR signals of (**1**) (Tables 1 and 2) at δc 95.7 and 176.4 in



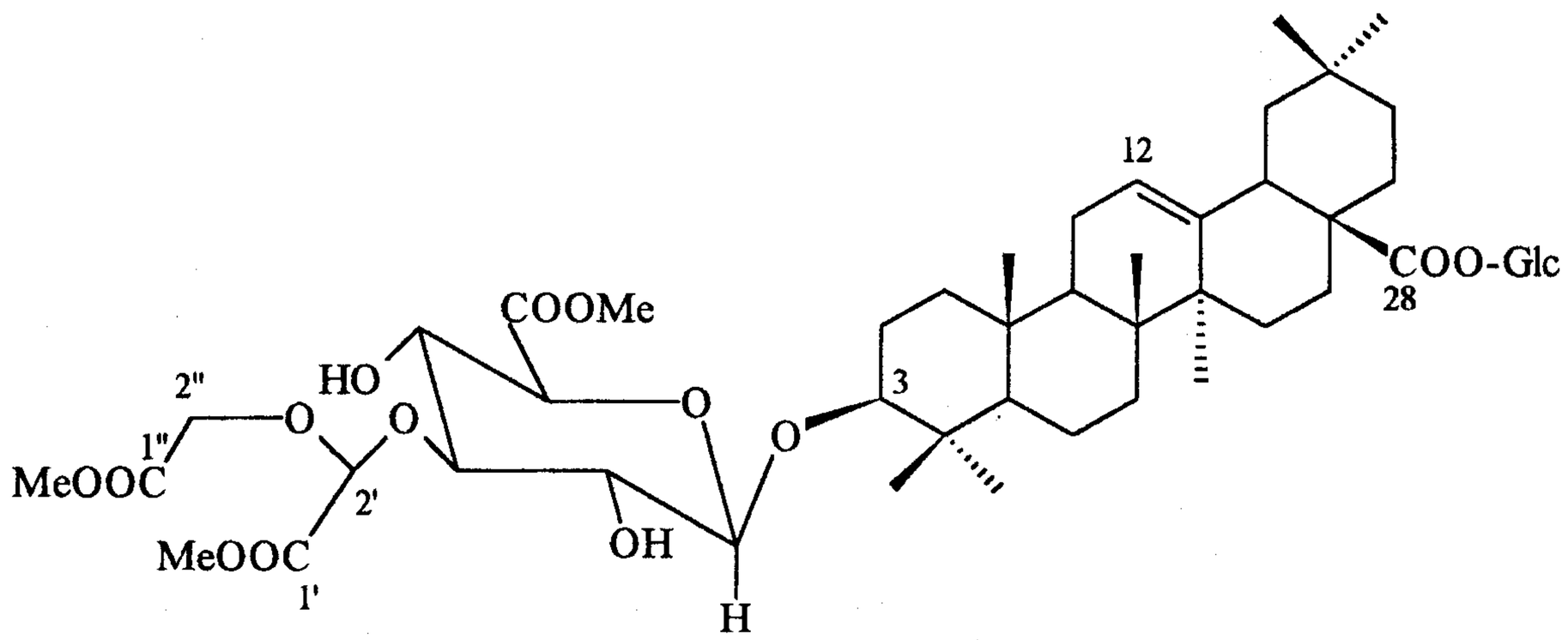
(1): R_1 = GlcA, R_2= Glc

(2): R_1= GlcA $\xrightarrow{2}$ Glc, R_2= Glc

(3): R_1= GlcA Me ester $\xrightarrow{3}$ Ara, R_2= Glc



(4): R_1= H, R_2= Glc **(5)**: R_1= H, R_2= Me **(6)**: R_1, R_2= Glc



(7)

Fig. 1: Structures of saponins (1-7)

Table 1: ^{13}C-NMR data of the aglycone part of Saponins (1-7), (100 MHz, C_5D_5N):

C	(1)	(2)	(3)	(4)	(5)	(6)	(7)
1	38.6	38.6	38.7	38.6	38.5	38.7	38.7
2	26.6	26.6	26.6	26.5	26.5	26.3	26.5
3	89.0	89.2	89.4	89.3	89.3	89.8	89.4
4	39.5	39.5	39.5	39.4	39.5	39.5	39.4
5	55.7	55.8	55.8	55.7	55.6	55.8	55.7
6	18.5	18.5	18.5	18.4	18.4	18.5	18.4
7	33.1	33.1	33.2	33.0	33.0	33.1	33.1
8	39.9	39.9	40.0	39.9	39.6	39.9	39.9
9	47.9	47.9	48.0	47.9	47.8	48.0	48.0
10	36.9	36.9	37.0	36.9	36.9	36.9	36.9
11	23.6	23.6	23.7	23.6	23.6	23.6	23.7
12	122.9	122.9	122.9	122.8	122.8	122.8	122.8
13	144.1	144.1	144.2	144.1	144.1	144.1	144.1
14	42.1	42.1	42.2	42.1	42.0	42.1	42.1
15	28.2	28.2	28.3	28.2	28.1	28.2	28.1
16	23.7	23.7	23.5	23.7	23.7	23.8	23.7
17	46.9	46.9	47.1	46.9	46.9	47.0	47.0
18	41.7	41.7	41.8	41.7	41.8	41.8	41.7
19	46.2	46.2	46.3	46.2	46.0	46.2	46.2
20	30.6	30.7	30.8	30.7	30.8	30.7	30.8
21	33.9	33.9	34.1	33.9	33.9	34.0	34.0
22	32.5	32.5	32.6	32.5	32.7	32.5	32.5
23	28.1	28.1	28.1	28.0	28.1	28.1	28.2
24	16.9	16.7	16.9	16.8	16.8	16.7	16.8
25	15.5	15.5	15.5	15.5	15.4	15.5	15.5
26	17.4	17.4	17.5	17.4	17.1	17.4	17.4
27	26.1	26.1	26.1	26.1	26.1	26.1	26.1
28	176.4	176.4	176.4	176.4	178.0	176.5	176.4
29	33.1	33.1	33.2	33.1	33.1	33.1	33.1
30	23.3	23.4	23.8	23.4	23.4	23.4	23.6
28-OMe					51.6		

Table 2: ^{13}C-NMR data of the sugar part of Saponins (**1-7**), (100 MHz, C_5D_5N):

C	(1)	(2)	(3)	(4)	(5)	(6)	(7)
GlcA							
1	107.2	105.3	105.9	106.7	106.8	104.1	106.8
2	75.1	82.8	74.5	74.5	74.6	78.8	74.8
3	78.1	77.1	85.8	84.7	84.7	82.6	84.2
4	73.4	73.1	72.9	71.9	71.9	72.5	71.1
5	77.8	77.4	76.8	76.8	76.8	76.5	76.8
6	172.8	172.5	170.2	170.2	170.2	170.1	170.3
Ara							
1			106.8				
2			71.3				
3			74.6				
4			69.3				
5			67.2				
Substituent (at C-3 of GlcA):							
1′				172.4	172.4	172.3	168.0
2′				73.8	73.8	74.1	99.4
3′				104.6	104.6	104.8	
1″				171.1	171.2	171.1	170.6
2″				63.9	64.0	64.6	63.1
OMe							
			52.1	52.1	52.1	52.1	52.1
				51.7	51.7	51.8	51.8
				51.3	51.3	51.4	51.5
ß-D-Glucose (at C-2 of GlcA):							
1		105.9				103.4	
2		77.9				78.2[a]	
3		78.2				78.2[a]	
4		71.7				72.3	
5		77.7				78.3[a]	
6		62.7				63.1	
ß-D-Glucose (at C-28 of aglycone):							
1	95.7	95.7	95.8	95.7		95.7	95.7
2	74.1	74.1	74.2	74.1		74.1	74.1
3	79.3	79.3	79.3	79.3		79.1	79.3
4	71.1	71.1	71.2	71.1		71.2	71.1
5	78.9	78.9	78.9	78.9		78.8	78.9
6	62.2	62.2	62.3	62.2		62.3	62.2

[a]Assignments may be interchangeable within each column.

comparison with those of free oleanolic acid[6,7] established the glycosylation at C-28 carboxyl group of the aglycone. This sugar was easily cleaved by alkaline hydrolysis using 5% aq. KOH and was identified ad D-glucose comparing with authentic material.

The second sugar moiety (D-glucuronic acid) showed a ß-ether linked anomeric proton in the ^{1}H-NMR at δ_H 4.99, 1H, *d*, *J*= 7.8 Hz with δc 107.2. It was attached to C-3 of the aglycone as established from ^{13}C-NMR data from the downfield shift of C-3 (*ca.* 9 ppm) and upfield shift of C-2 (*ca.* 2 ppm) in comparison with those of oleanolic acid.[6,7] As such saponin (**1**) was identified as 3-*O*-ß-D-glucuronopyranosyl, 28-*O*-ß-D-glucopyranosyl oleanolic acid (chikusetsusaponin IVa).[8,9]

Acid hydrolysis of saponins (**2-4**), (**6**) and (**7**) afforded aglycone which was also identified as oleanolic acid as previously mentioned.

The FAB mass and NMR spectral data of saponin (**2**) exhibited the presence of one extra glucose unit than that of (**1**) with δc 105.9 and δ_H 5.38, 1H, *d*, *J*= 7.8 Hz. Its linkage at C-2 of glucuronic acid was deduced from the downfield shift of glucuronic acid C-2 to δc 82.8 and upfield shift of C-1 to 105.3 when compared with those of (**1**) at 75.1 anbd 107.2 respectively. Therefore, saponin (**2**) can be characterized as 3-*O*-ß-D-glucopyranosyl-(1→2)-ß-D-glucuronopyranosyl, 28-*O*-ß-D-glucopyranosyl oleanolic acid (chikusetsusaponin IV).[8,10]

Comparison of NMR spectra of saponin (**3**) with that of (**1**) showed the presence of an extra pentosyl unit which was suggested to be α-L-arabinopyranosyl moiety from the data at δc 106.8 (with δ_H 5.31, 1H, *d*, *J*= 7.0), 71.3, 74.6, 69.3 and 67.2. Its location at C-3 of glucuronic acid was established from the downfield shift of glucuronic acid C-3 to δc 85.8 comparing with that of (**1**) at 78.1. From the results of acid hydrolysis of (**3**), the sugar residues were identified as glucuronic acid methyl ester, arabinose and glucose by comparison with authentic samples. From the above findings and by comparison with the data reported earlier, the structure of saponin (**3**) was assigned as 3-*O*-α-L-arabinopyranosyl-(1→3)-ß-D-glucuronopyranosyl, 28-*O*-ß-D-glucopyranosyl oleanolic acid methyl ester (momordin IIa methyl ester).[9]

The molecular formula of compound (**4**) was assigned from ^{13}C, DEPT ^{13}C-NMR and FAB-MS spectral data as $C_{50}H_{78}O_{20}$. The negative ion FAB-MS spectrum of (**4**) showed a molecular ion peak at m/z: $[M-H]^-$ 997 which was greater than that of (**1**) by 204 mass unit. The ^{1}H-NMR (experimental section) and ^{13}C-NMR (Tables 1 and 2) spectral data of (**4**) indicated the presence of a 3-*O*-ß-D-glucurono-pyranosyl, 28-*O*-ß-D-glucopyranosyl oleanolate methyl ester and an acidic substituent composed of tartroaldehydic acid and glycolic acid at the C-3 hydroxyl group of the 3-*O*-glucuronic acid moiety from the signals at δc 172.4 (C-1′), 73.8 (C-2′), 104.6 (C-3′), 171.1 (C-1″) and 63.9 (C-2″) with δ_H 5.11 (1H, *d*, *J*= 3.2 Hz, H-2`), 6.0 (1H, *d*, *J*= 3.2 Hz, H-3`), and the ABq system of H-2″ at 4.70 (1H, *d*, *J*= 16.6 Hz, H-2″a) and 5.14 (1H, *d*, *J*= 16.6 Hz, H-2″b). Its connectivity to the 3-*O*-glucuronic acid was suggested from the downfield of glucuronic acid C-3 to δc 84.7 when compared with compound (**1**) at 78.1. The measurment of 2D NMR spectra viz. H-H COSY, C-H COSY and HMBC spectral analysis established the structure of this acidic substituent and confirmed its connectivity to the 3-*O*-glucuronic acid as shown in Fig. 2. From the above mentioned data, saponin (**4**) was unambiguously identified as betavulgaroside III methyl ester which has been isolated before as a free acid from *Beta vulgaris* L. (Chenopodiaceae).[11]

The acid hydrolysis of compound (**5**) using 10% aqueous HCl afforded oleanolic acid and glucuronic acid methyl esters which were identified by comparison with authentic samples and from their spectral data. The ^{1}H and ^{13}C-NMR spectra of (**5**) were similar to those of (**4**) except for absence of 28-*O*-ß-D-glucopyranosyl unit, instead of which a signal corresponding to 28-COOMe has been displayed as a result of methylation with diazomethane. Therefore, saponin (**5**) was unequivocally identified as betavulgaroside IV methyl ester which has been isolated before as a free acid from *Beta vulgaris* L.[11]

The FAB mass and NMR spectral data of saponin (**6**) exhibited the presence of an

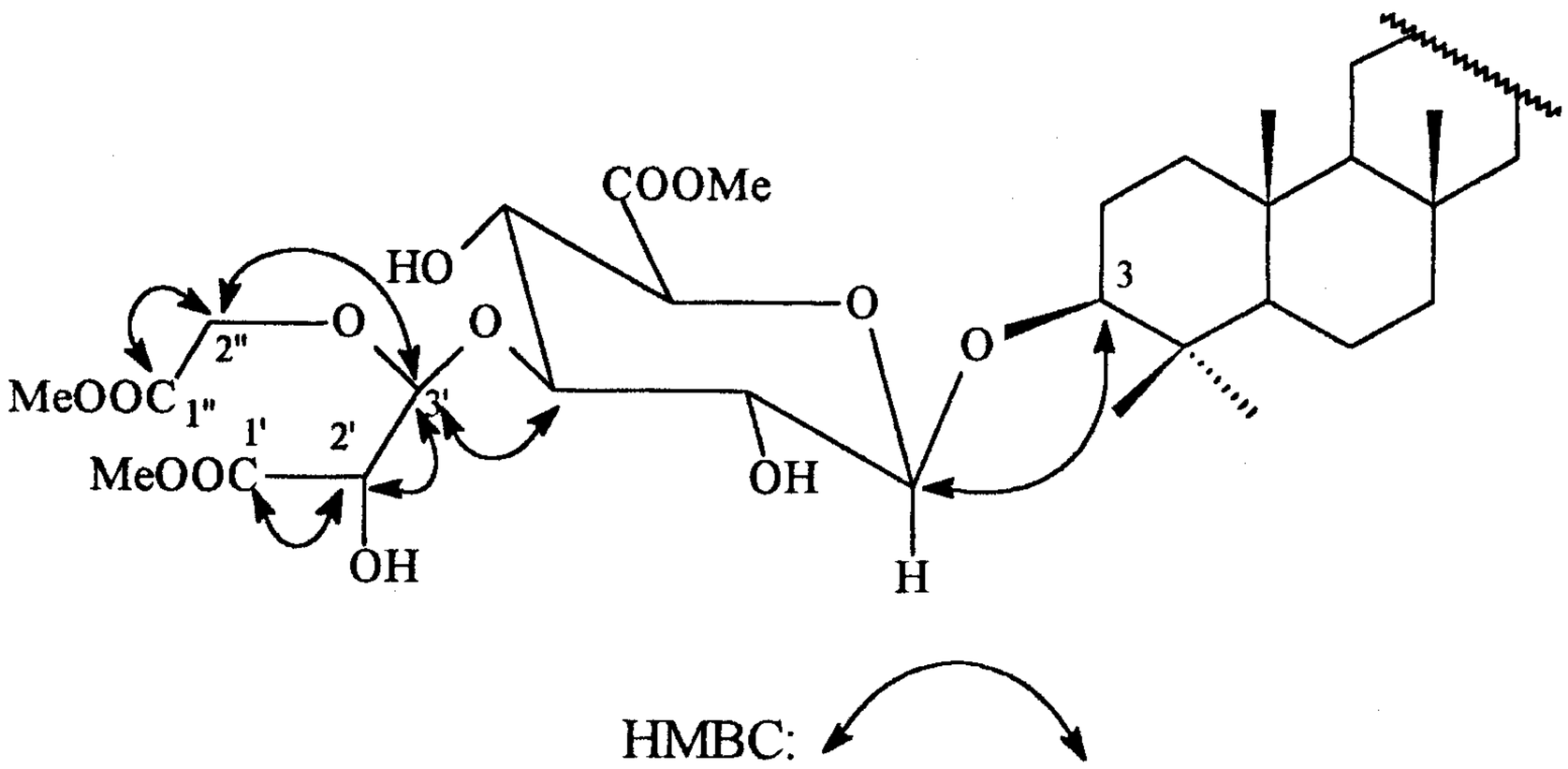


Fig. 2: Important HMBC corrrelations of saponin (**4**)

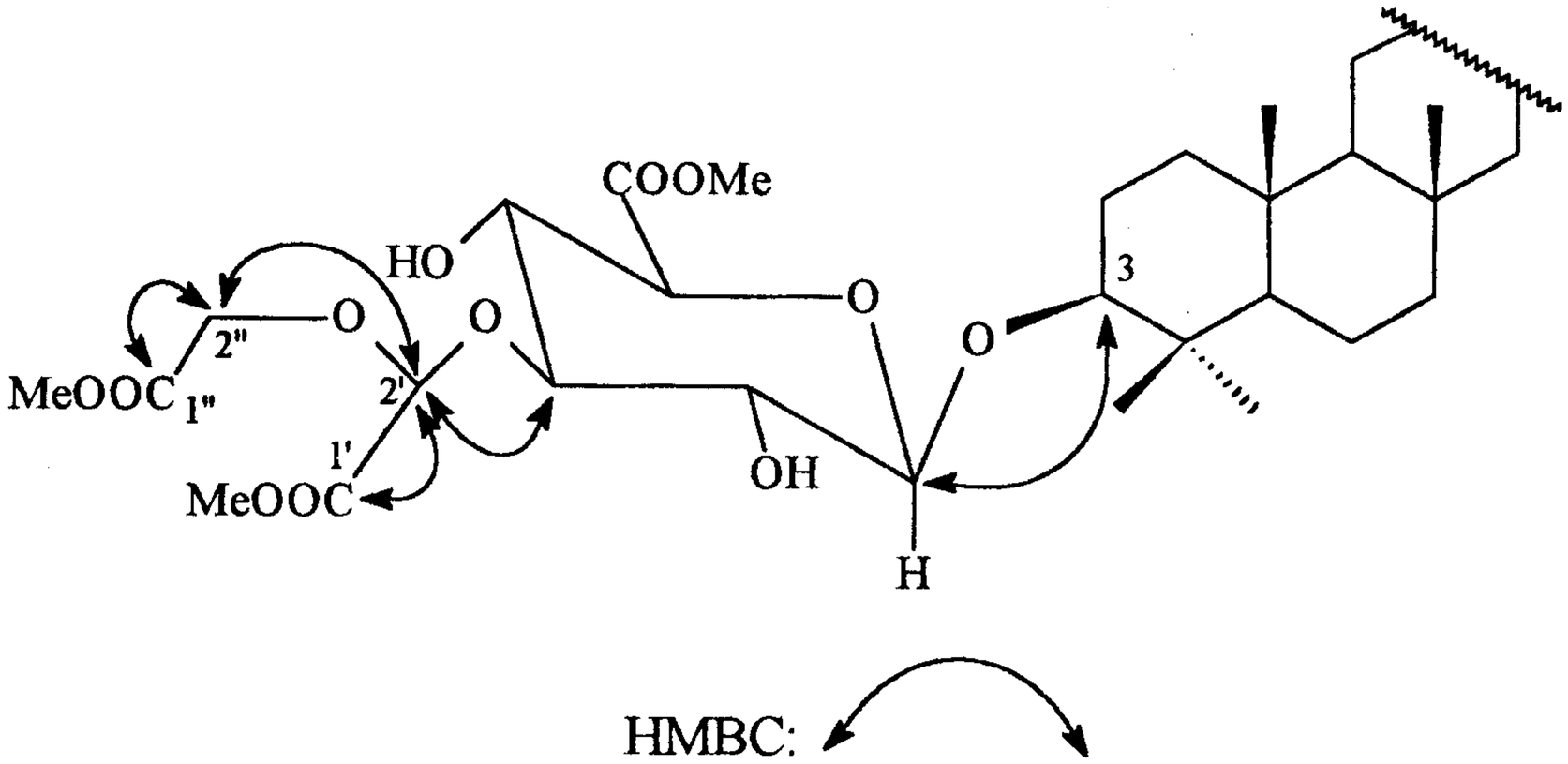


Fig. 3: Important HMBC corrrelations of saponin (**7**)

additional one glucose unit than that of (**4**) with δc 103.4 and δ_H 5.46, 1H, *d*, *J*= 7.6 Hz. Its location at C-2 of glucuronic acid was deduced from the downfield shift of glucuronic acid C-2 to δc 78.8 and upfield shifts of glucuronic acid C-1 and C-3 to 104.1 and 82.6 respectively when compared with those of (**4**) at 74.5, 106.8 and 84.7 respectively. Consequently, saponin (**6**) can be characterized as betavulgaroside V methyl ester which has been isolated before as a free acid from *Beta vulgaris* L.[12]

The molecular formula of compound (**7**) was assigned from ^{13}C, DEPT ^{13}C-NMR and FAB-MS spectral data as $C_{49}H_{76}O_{19}$. The negative ion FAB-MS spectrum of (**7**) showed a molecular ion peak at m/z: $[M-H]^-$ 967 which was lower than that of (**4**) by 30 mass unit which was attributed to lack of -CHOH function in (**7**). The ^1H-NMR (experimental section) and ^{13}C-NMR (Tables 1 and 2) spectral data of (**7**) indicated also the presence of a 3-*O*-ß-D-glucuronopyranosyl, 28-*O*-ß-D-glucopyranosyl oleanolate methyl ester and an acidic substituent as expected composed of 2´-(2"-O-glycolyl)-glyoxylyl- at the C-3 hydroxyl group of the 3-*O*-glucuronic acid moiety from the signals at δc 168.0 (C-1´), 99.4 (C-2´), 170.6 (C-1") and 63.1 (C-2") with δ_H 6.24 (1H, *s*, H-2´) and the ABq system of H-2" at 4.90 (1H, *d*, *J*= 16.3 Hz, H-2"a) and 5.06 (1H, *d*, *J*= 16.3 Hz, H-2"b). Its linkage to the 3-*O*-glucuronic acid was suggested from the downfield of C-3 of glucuronic acid moiety of (**7**) to δc 84.2 in comparison with that of compound (**1**) at 78.1. The aforementioned results were confirmed by measurment of C-H COSY and HMBC spectral analysis (Fig. 3). Accordingly, the structure of saponin (**7**) can be formulated as 3-O-{2´-(2"-*O*-glycolyl)-glyoxylyl-ß-D-glucuronopyranosyl}28-*O*-ß-D-glucopyranosyl oleanolic acid methyl ester. This compound has been isolated earlier as a free acid from *Pisonia umbellifera* Seem. (Nyctaginaceae)[13] and this represents its isolation for the first time from Chenopodiaceae.

It has been reported that the aforementioned triterpenoidal *seco*-glycosides (**4-7**) may originate from normal pentose glycosides through a series of oxidative ring cleavage of pentose residues.[13,14]

It is also noteworthy to mention that the rare triterpenoidal *seco*-glycosides has been so far isolated from three other sources; *Achyranthes fauriei* (Amaranthaceae),[15] *Beta vulgaris* (Chenopodiaceae)[11,12,14] and *Pisonia umbellifera* (Nyctaginaceae)[13] which are belong to the order of Centrospermales[13] and this represents their fourth report.

This study also represents the first report of isolation of saponins (**1-7**) from genus *Kochia* (Chenopodiaceae) and compound (**7**) is first reported from family Chenopodiaceae.

The occurrence of saponins (**4-6**) in *Kochia indica* and *Beta vulgaris* (Chenopodiaceae) is presumably a chemotaxonomic marker of this family.

It was also previously reported that betavulgarosides II and IV showed strong hypoglycemic activity on the elevation of plasma glucose in the oral D-glucose tolerance test in rats, moreover betavulgaroside III also showed inhibitory activity but was weaker than those of II and IV.[11]

Work is in progress for studying the biological activities of the isolated compounds as well as the isolation of the constituents of the 50% methanolic eluate.

Acknowledgments

The first two authors are grateful to the Research Center of Molecular Medicine of the Hiroshima Univ. School of Medicine, Japan for carrying out the spectral analysis.

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