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A Novel Prosapogenin from the Methanolyzate of *Melandrium* Crude Saponins

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Abstract—Two compounds were isolated from the methanolyzate of the butanol-soluble fraction obtained from the whole plants of *Melandrium firmum* (Caryophyllaceae) and identified as 3-β-D-glucuronopyranosylmelandrigenin methyl ester and 2β, 21β-dihydroxy-16, 23-dioxo-28-norolean-13(18)-ene.

Keywords—*Melandrium firmum* • Caryophyllaceae • 3-β-D-glucuronopyranosylmelandrigenin methyl ester • 3β, 21β-dihydroxy-16, 23-dioxo-28-norolean-13(18)-ene

Melandrium firmum (caryophylaceae) has been known as a saponin-bearing plant[1] and several sapogenins were isolated such as gypsogenin, gypsogenic acid, quillaic acid and melandrigenin recently.[2,3] This paper deals with the isolation and characterization of a prosapogenin(**1**) and a minor sapogenin(**5**). Acid hydrolysis of a butanol-soluble fraction in methanol and column chromatography yielded compound **1** and **5** in addition to the previously reported sapogenins.[2,3]

Compound **1**, mp 286°, gave a yellow coloration in the Liebermann-Burckard test. Its IR spectrum showed the presence of a hydroxy group (3440 cm^{-1}), three kinds of carbonyl function (1750, 1715 and 1705 cm^{-1}), a double bond (1660 cm^{-1}) and a glycoside bond (1000~1100 cm^{-1}). The glycosidic nature of **1** was clearly indicated by many resonances in the region of δ 3.1~4.2 ppm in its ^{1}H-NMR spctrum and δ 71.3~75.8 ppm in its ^{13}C-NMR spectrum.

Its ^{1}H-NMR exhibited six tertiary methyl signals at δ 0.77~0.98, one olefinic proton at δ 5.38, accountable for a trisubstituted double bond very similar to the corresponding signal in β-amyrins, and one aldehyde proton at δ 9.33, one methoxy carbonyl protons at δ 3.65 and an anomeric proton at δ 4.14 ppm (d, J=7.5 Hz), indicating the presence of one sugar residue. The^{13}C-NMR spectrum of **1** showed 36 carbon resonances including those for one aldehyde (δ 206.4), one ketone (δ 212.7), one methoxy carbonyl function (δ 169.2 and 51.6), six secondary OH groups (δ 71.3~80.7), one anomeric carbon (δ 103.2), six methyls (δ9.4~18.2, 24.9 and 29.1) together with a trisubstituted double bond (δ 116.9 and 141.8), which strongly suggested the nortriterpene skeleton with 17α-hydrogen-*trans*-D/E ring junction.[3,4]

Acid hydrolysis of **1** afforded an aglycone(**3**), identified as melandrigenin from its MS spectral data and by ^{1}H-and ^{13}C-NMR spectra of its acetate(**4**).[3] As a sugar, glucuronic acid was detected in the hydrolysate by TLC.

The position of attachment of the sugar to the aglycone was established as C-3 position by comparison of ^{13}C-NMR spectra of the prosapogenin acetate(**2**) and melandrigenin acetate(**4**). All the chemical shift values of both acetates, but those for C-3 were almost identical with

each other.

β-Configuration of glycosidic linkage was deduced from the coupling constant of the anomeric proton and the chemical shift value of the anomeric carbon. Therefore, the structure of **1** was elucidated to be 3-O-β-D-glucuronopyranosyl melandrigenin methyl ester. Compound **1** can be considered to be a prosapogenin formed during methanolysis of the saponin, since it is known that the glucuronosidic bond are cleaved with greater difficulty than glycosidic bonds of aldoses.[5]

Compound **5**, mp 252~256°, gave negative result in the Liebermann-Burchard test. Its IR spectrum showed the presence of a hydroxy group(3, 400 cm^{-1}), two kinds of carbonyl functions (1735 and 1660 cm^{-1}) and a double bond (1640 cm^{-1}) and its UV spectrum showed simple carbonyl absorption with 285 nm. It gave a diacetate(**6**) on acetylation. The ^{1}H-NMR spectrum of **6** showed six tertiary methyl signals at δ 0.87~1.06, which was reminiscent of the oleanane type triterpene, two acetoxy singlets at δ 1.95 and 2.03, two oxymethine protons at δ4.79(1H, dd, J=5.5 and 12.0 Hz, H-21) and 4.93(1H, dd, J=7.5 and 9.0 Hz, H-3) and one aldehyde proton at δ 9.26. However, no olefinic proton was observed, suggesting that compound **5** seemed to be an olean-13(18)-ene.

The ^{13}C-NMR spectrum of **6** not only showed the presence of 33 carbons, indicating a nor-triterpenoid, but also showed the presence of one aldehyde (δ 204.2), one ketone (δ 208.7), two secondary OH groups (δ 73.3 and 78.7) and a tetrasubstituted double bond (δ 124.9 and 132.8), which supported the location of the double bond.

The MS fragmentation patterns of **5**, clearly showed an olean-13(18)-ene skeleton and the presence of one hydroxy and one aldehyde on rings A/B and of the second hydroxy and one ketone on rings D/E, lacking one methyl group.[6]

Signals adjacent to the hydroxy groups were fundamentally identical with those of H-3α and H-21α of **4** (see Experimental). Moreover, the carbon signals for rings A, B and E of **6** are similar to those of **4**.

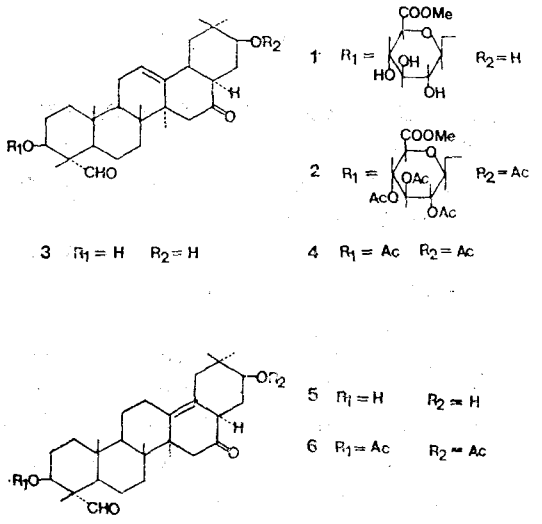
In the light of the above observations, the structure of **5** was established as 3β, 21β-dihydroxy-16, 23-dioxo-28-norolean-13(18)-ene. However, compound **5** was not assumed to be a genuene sapogenin but an artefact formed from melandrigenin by an acid-induced isomerization.

Experimental

General procedures—Melting points were determined on a Mitamura-Riken apparatus and uncorrected. IR spectra were recorded on a Perkin-Elmer 283B spectrophotometer. UV spectra were recorded on a Gilford system 2, 600 UV-VIS spectrophotometer. NMR spectra were obtained on a Varian FT-80A spectrometer. EIMS spectrum were determined on a Hewlett-Packard 5985B GS/MS system.

Extraction and isolation—The powdered dry whole plants of *M. firmm* were refluxed with MeOH. The MeOH extract was partitioned with hexane, $CHCl_3$, EtOAc and BuOH, successively. The BuOH-soluble fraction was hydrolysed with 5% H_2SO_4 in MeOH for 5 hr, added to water. The precipitate was filtered, washed with water, and dried to give a brown solid, which was chromatographed over an SiO_2 column with the solvent of $CHCl_3$-MeOH (gradient). The fractions were monitored by TLC and from the collected fraction compound **1** and **2** were obtained together with the preriously isolated sapogenins.[2,3]

Compound 1—crystallized from MeOH as needles, mp. 286°, UV λ_{max}^{MeOH} nm(log ε), 298.5 (2.51); IR and ^{1}H-NMR, see text; ^{13}C-NMR, see Table I.



Compound 5—crystallized from MeOH as needles, mp. 252~256°, UV λ_{max}^{MeOH} nm(log ε), 285.0(2.15); MS(70ev) m/z (rel. int.): 456(M^+, 43.6), 234(D/E rings via cleavage of 8-14 and 9-11 bonds, 6.4), 221(A/B rings, 30.5), 220 (D/E rings via cleavage of 8-4 and 11-12 bonds, 18.5), 203(221-H_2O, 90.5); IR and ^{1}H-NMR, see text; ^{13}C-NMR, see Table I.

Acetylation of compound 1—A sample of **1** (100 mg) was treated with Ac_2O/pyridine(1 : 1) at room temperature overnight. Workup in the usual way, followed by column chromatography (hexane-EtOAc, 1% to 10%) afforded **2**, as an amorphous powder, ^{1}H-NMR ($CDCl_3$) δ: 1.87(6H, s, acetate×2), 1.97(3H, s, acetate), 2.01(3H, s, acetate); ^{13}C-NMR, see Table I.

Acid hydrolysis of compound 1—Compound **1**(50 mg) was refluxed with 5% H_2SO_4 in 60% dioxane for 5 hr, added to crushed ice. The precipitate was filtered, washed with water, crystallized from MeOH to give **3** as needles, mp. 294°, MS(70eV), m/z(rel. int.): 456(M^+, 30.5), 234(RDA with rings D/E, 65.2), 221 (RDA with rings A/B, 12.9). Acetate(**4**), mp. 267~268°; MS(70eV) m/z (rel. int.): 480(M^+-HOAc, 59.4), 276(RDA with rings D/E, 29.5); ^{1}H-NMR($CDCl_3$) δ: 0.83~1.05(Me×6),

Table I. ^{13}C-NMR chemical shifts of compound **1**, **2**, **4** and **6** (20 MHz in $CDCl_3$)

Carbon No.	1*	2	4	6
1	37.1	37.8	37.6	38.0
2	24.0	24.6	22.4	22.5
3	80.7	83.1	73.3	73.3
4	54.2	54.7	54.1	54.2
5	46.6	48.0	48.0	48.2
6	19.6	20.3	20.5	20.7
7	31.6	32.3	32.3	33.7
8	38.4	39.0	39.0	43.4
9	46.3	47.1	47.1	51.1
10	35.6	36.2	36.1	36.3
11	22.6	23.2	23.2	22.0
12	116.9	118.2	118.1	20.6
13	141.8	141.5	141.8	124.9
14	42.2	42.6	42.6	41.0
15	43.4	42.6	42.7	43.7
16	212.7	212.3	212.1	208.7
17	47.7	48.8	48.0	52.0
18	35.6	36.2	36.1	132.8
19	42.2	43.7	42.8	38.0
20	35.0	34.5	34.5	36.8
21	74.8	78.1	78.1	78.7
22	31.2	27.7	27.7	24.1
23	206.4	205.8	203.9	204.2
24	9.4	9.8	9.4	9.2
25	15.0	15.4	15.5	16.6
26	16.3	16.8	16.8	17.7
27	24.9	25.7	25.7	24.0
28				
29	29.1	28.8	28.8	27.9
30	18.2	19.2	19.3	19.5
1'	103.2	101.9		
2'	73.2	71.0		
3'	75.8	72.4		
4'	71.3	69.5		
5'	75.3	72.0		
6'	169.2	167.0		
OAc		20.0	20.8	21.0×2
		20.3×2	20.9	
		20.9		
		169.1×2	169.8	169.8×2
		169.8	170.2	
		170.4		
OCH_3	51.6	52.6		

* Recorded in DMSO-d_6

1.93(3H, s, acetate), 2.02(3H, s, acetate), 4.57(1H, dd, J=4 and 12 Hz, H-21), 4.98 (1H, dd, J=7 and 9 Hz, H-3), 5.45(1H, m, H-12), 9.28(1H, s, CHO); ^{13}C-NMR, see Table I.

Acetylation of 5—A sample of 5(70 mg) was treated with Ac_2O/pyridine(1 : 1) at room temperature. Workup in the usual way afforded 6, as an amorphous powder, ^{1}H-NMR, see Text; ^{13}C-NMR, see Table I.

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