Phytochemical Study on *Melandrium Firmum*

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Abstract □ The aglycones of saponins isolated from *Melandrium firmum* were identified as gypsogenin, gypsogenic acid and quillaic acid.

Keywords □ *Melandrium firmum*, Caryophyllaceae, Triterpenoid, Gypsogenin, Gypsogenic acid, Quillaic acid, ^{13}C NMR.

The whole plants of *Melandrium firmum* (Caryophyllaceae), which is a biennial herbaceous plant, have been used for a Chinese crude drug "Wang-pu-liu-hsing" as a remedy against anuria, breast cancer, gonorrhea, and diseases of lactation.[1] It was recently found that a methanol extract caused a significant prolongation of hexobarbital-induced sleeping time, elevation of serum transaminase activities and severe histopathological changes in the liver in mice.[2] The saponin fraction obtained through the systematic fractionation of the methanol extract monitoring by bioassay showed toxiciy.[3] This paper deals with the isolation and characterization of the sapogenins.

Column chromatography on silica gel of the acid hydrolysate of the butanol soluble fraction of methanol extracts, eluting with 5 % methanol in chloroform furnished five sapogenins.

Compound 1, mp 243~4°, $[\alpha]_{20}^D + 65.4°$, showed the positive Libermann-Burchard test and strong absorption bands at 3450(OH), 1720 (aldehyde), 1690 (free acid), 1660 and 810cm^{-1} (trisubstituted double bond) in its IR spectrum.

The ^{1}H NMR and ^{13}C NMR data (DMSO-d_6) showed the presence of one proton, each of olefins (δ 5.17, m), oxymethine (δ 3.62, m) and aldehyde (δ 9.25, s), two olefinic carbons (δ 121.2, d and 143.8, s) and one carbon, each of oxymethine (δ 70.47, d), acid (δ 178.3, s) and aldehyde (δ 206.8, d).

Methylation with CH$_2$N$_2$ of Compound 1 and subsequent acetylation with acetic anhydride-pyridine gave a methylester, mp 184~186° and a methylester acetate, mp 186~8°, respectively.

The MS spectrum of Compound 1 showed a molecular ion at m/z 470 and retro-Diels-Alder fragment ions at m/z 248 and 221, suggesting that it is a compound having Δ^{12}–oleane skeleton with one carboxyl group at rings D and E and one hydroxyl group and one aldehyde group at rings A and B.[4]

The ^{1}H NMR spectrum of the methylester acetate showed six angular methyl signals at δ 0.71(3H), 0.88(3H), 0.09(3H), 0.96(3H), 1.06(3H) and 1.12(3H), one acetyl signal at 1.93(3H), one carbomethoxyl signal at 3.59(3H), a double doublet centered at 4.95 (1H, J=8 and 10Hz) due to H-3, a multiplet centered at 5.27 (1H), due to H-12 and a singlet at 9.25 (1H) for an aldehyde group.

The formation of a monobromo-γ-lactone by treatment of Compound 1 with Br$_2$-HOAc and the appearance in NMR of Compound 1-methylester acetate of the highest angular methyl

signal in upfield region from 0.77 ppm strongly supported the presence of a carboxyl group at C-17 in Compound **1**.[5]

Treatment of Compound **1** methylester with LiAlH₄ gave a triol (**5**), mp 248∼250° which was identified as 23,28-dihydroxy-β-amyrin by direct comparison with an authentic reduction product prepared from hederagenin (**4**). From the above data, Compound **1** was characterized as 3β-hydroxy, 23-oxo-olean-12-en-28-oic acid (gypsogenin).

Compound **2**, mp 298°, $[\alpha]_{20}^D + 80.2°$, also showed positive Liebermann-Burchard test. Its IR, ¹H NMR and ¹³C-NMR spectra were similar to those of gypsogenin but showed the absence of an aldehyde function. Methylation with CH₂N₂ of Compound **2** and subsequent acetylation with acetic anhydride-pyridine gave a dimethylester, mp 251∼253° and a dimethylester acetate, mp 174∼6°, respectively.

The MS spectrum of Compound **2** showed a molecular ion at *m/z* 486 and RDA fragment ions at *m/z* 248 and 237, suggesting that it is a compound having Δ¹²-oleanene skeleton with one carboxyl group at rings D and E and one hydroxy group and another carboxyl group at rings A and B.[3]

The ¹H NMR spectrum of the dimethylester acetate showed six angular methyl signals at δ 0.70 (3H), 0.91(9H), 1.13(3H) and 1.18(3H), one acetyl signal at 1.96(3H), two carbomethoxyl signals at 3.61(3H) and 3.65(3H), a double boublet centered at 5.15 (1H, J=8 and 10 Hz) due to H-3 and a multiplet centered at 5.27 (1H) due to H-12.

The point of attachment of the two carboxyl group was established by the formation of a triol (**5**), mp 248∼50°, by treatment of Compound **2**-dimethylester with LiAlH₄.

From the above data, the structure of Comp-

ound **2** was elucidated as 3β-hydroxy-olean-12-ene-23,28-dioic acid (gypsogenic acid). Compound **3**, mp 266∼70°, $[\alpha]_{20}^D + 44°$, also showed positive Liebermann-Burchard test and its IR and ¹H NMR spectra were very similar to those of Compound **1**, except the appearance of a signal at δ 4.31. Methylation with CH₂N₂ of Compound **3** and subsequent acetylation with acetic anhydride-pyridine gave a methylester, mp 200∼5° and two products, a methylester monoacetate, mp 234∼6°, and a methylester diacetate, mp 170∼2°, respectively. The monoacetate was easily converted to the diacetate by boiling with acetic anhydride-pyridine. This result indicated the presence of a sterically hindered hydroxyl group in Compound **3**.

The MS spectrum of Compound **3** showed a molecular ion at *m/z* 486 and RDA fragment ions at *m/z* 264 and 221, suggesting that it is a compound having Δ¹²-oleanene skeletone with one hydroxyl group and one carboxyl group at rings D and E and another hydroxyl group and one aldehyde group at rings A and B.[3]

The ¹H NMR spectrum of the methylester diacetate showed six angular methyl signals at δ 0.72∼1.26, two acetyl signals at 1.93 (3H) and 2.08(3H), one carbomethoxyl signal at 3.62(3H), a doudle doublet centered at 4.95 (1H, J=8 and 10Hz) due to H-3, a multiple centered at 5.40(1H) due to H-12 and a singlet at 9.25(1H) for an aldehyde group. The second proton α to an acetoxyl group appeared at 5.51(1H) as a multiplet.

Observed values in Hz (85.6, 78.4, 57.6, 100.8, 73.6 and 78.4) of the chemical shifts for the C-methyl groups of the methyl diacetate are in excellent agreement with the calculated values (83.7, 77.5, 53.6, 98.5, 74.5 and 76.0 for Me 24,25,26,27,29 and 30, respectively) for 3β, 16α-diacetoxy, 23-oxo-olean-12-en-28-

Table I: ^{13}C NMR chemical shifts of oleanenes (1, 2, 4 & 5) in DMSO-d[6] and their methyl acetates (in parentheses) in CDCl[3]

Carbon	1	2	4	5
C- 1	37.6(37.9)	38.0(38.0)	37.8	37.9
C- 2	26.8(22.6)	26.5(21.1)	26.4	26.3
C- 3	70.5(73.5)	73.8(77.3)	70.7	70.6
C- 4	54.9(54.3)	52.6(52.2)[d]	41.6	41.6[b]
C- 5	46.9(48.0)	50.7(52.0)[d]	47.0	46.9
C- 6	20.1(20.4)	20.5(12.0)[e]	17.4	17.3
C- 7	32.0(32.4)	32.0(32.4)	31.9[a]	31.7
C- 8	39.2(39.8)	39.2(39.8)	38.8	39.1
C- 9	46.4(47.7)	47.2(47.8)	46.7	46.5
C-10	35.4(35.9)	36.9(36.5)	36.2	36.3[c]
C-11	22.8(23.4)	22.8(23.4)	22.8	22.8
C-12	121.2(121.9)	121.4(122.0)	121.4	121.3
C-13	143.8(144.0)	143.7(143.9)	143.7	144.1
C-14	41.4(41.8)	41.3(41.8)	41.3	41.1
C-15	27.1(27.8)	27.2(27.8)	27.1	25.0
C-16	22.6(23.1)	22.6(23.1)	22.8	21.6
C-17	45.4(46.8)	45.4(46.8)	45.3	36.1[c]
C-18	40.8(41.5)	40.8(41.4)	40.7	41.6[b]
C-19	45.7(46.0)	45.7(46.0)	45.6	46.2
C-20	30.2(30.7)	30.2(30.7)	30.1	30.4
C-21	33.3(34.0)	33.3(34.0)	33.3	33.7
C-22	32.0(32.1)	32.0(32.3)	31.9[a]	30.6
C-23	206.8(204.2)	178.4(176.7)	65.1	65.0
C-24	8.8(9.5)	11.0(11.9)[e]	12.1	12.2
C-25	15.1(15.5)	15.3(15.7)	15.3	15.4
C-26	16.7(16.9)	16.6(16.8)	16.7	16.3
C-27	25.5(25.9)	25.5(25.9)	25.4	25.4
C-28	178.3(178.0)	178.3(178.1)	178.2	67.2
C-29	32.7(33.0)	32.2(33.0)	32.6	32.8
C-30	23.3(23.6)	23.3(23.6)	23.2	23.3
COOMe	(51.3)	(51.2 & 51.4)		
COMe	(20.8)	(20.9)		
COMe	(169.9)	(169.9)		

a, b; overlapped   c, d, e; may be reversed

oic acid methyl ester. Consideration of these data indicated a structure corresponding to quillaic acid for Compound 3.
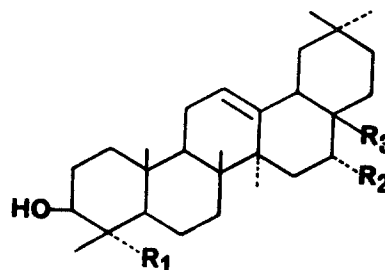
	R[1]	R[2]	R[3]
1	CHO	H	COOH
2	COOH	H	COOH
3	CHO	OH	COOH
4	CH[2]OH	H	COOH
5	CH[2]OH	H	CH[2]OH

A direct comparison (mmp, co-TLC, MS and NMR as methyl diacetate) with an authentic sample kindly provided by Shionogi Co. Japan confirmed the identity of these two terpenoids.

EXPERIMENTAL METHODS

The mps were taken on Mitamura-Riken apparatus and are uncorrected. The IR spectra were determined in KBr tablets on a Perkin-Elmer Model 281—283 IR spectrometer. ^{1}H-NMR (80MHz) and ^{13}C-NMR (20MHz) were recorded with a Varian FT-80A in DMSO-d[6] (free) and CDCl[3] (methylacetate) containing TMS as an internal standard and chemical shifts are given as δ (ppm). Mass spectra were obtained with Hewlett Packard Model 5985B GC/MS spectrometer. Optical rotations were measured on a Rudolph Autopol[R] III automatic polarimeter.

Isolation of the sapogenins

The powdered whole plants of *M. firmum* (4.75kg) was refluxed with MeOH. The MeOH extract (190 g) was partitioned with hexane, CHCl[3], ethylacetate and BuOH successively.

The hepatotoxic BuOH soluble fraction (55 g) was hydrolized with 5 % H[2]SO[4] for 5hr. The

precipitate was filtered, washed with water and dried to give a brown solid, which was chromatographed over SiO_2 column eluting chloroform-MeOH (95:5) to give Compound *1*, *2* and *3*.

Compound 1 (gypsogenin) Needles from MeOH, mp 243~4°, $[\alpha]_D^{20}+65.4°$ (MeOH:c 0.425) LB : positive.

IR ν_{max}^{KBr}(cm^{-1}) : 3450(OH), 1720(aldehyde), 1690(acid), 1660, 810(trisubstituted double bond); ^{1}H NMR (DMSO-d$_6$) δ : 0.72~1.13(Me ×6), 3.62(1H, t, H-3), 5.17(1H, m, H-12), 9.25(1H, s, CHO); MS(*m/z*) (rel. int.); 470 [M]$^+$ (1.8), 455[M-CH$_3$]$^+$ (0.4), 248[D/E ring]$^+$ (97.1), 221[A/B ring]$^+$ (5.2), 203[248-COOH]$^+$ (100).

Compound 2 (gypsogenic acid) Needles from MeOH mp 298°, $[\alpha]_D^{20}+80.2°$ (MeOH: c 0.5), LB : positive.

IR ν_{max}^{KBr}(cm^{-1}) : 3400(OH), 1700(acid), 1650, 816(trisubstituted double bond); ^{1}H NMR(DMSO-d$_6$) δ : 0.71~1.10(Me×6), 3.74(1H, m, H-3), 5.17(1H, m, H-12); MS(*m/z*)(rel. int.): 486[M]$^+$ (0.8), 440[M-(COOH+H)]$^+$ (1.2), 248[D/E ring]$^+$ (96.3), 237[A/ B ring]$^+$ (5.8), 219[237-H$_2$O]$^+$ (9.6), 203[248-COOH]$^+$ (100).

Compound 3 (quillaic acid) Needles from MeOH, mp 266~70°, $[\alpha]_D^{20}+44°$ (MeOH: c 0.23), LB : positive. IR ν_{max}^{KBr}(cm^{-1}) : 3400(OH), 1720(aldehyde), 1690(acid), 1660, 810(trisubstituted double bond); ^{1}H NMR(DMSO-d$_6$) δ : 0.68~1.33(Me×6), 3.60(1H, m, H-3), 4.31 (1H, m, H-16), 5.21(1H, m, H-12), 9.25(1H, s, CHO); MS(*m/z*)(rel. int.) : 486[M$^+$] (1.8), 486[M-H$_2$O]$^+$ (1.4), 264[D/E ring]$^+$ (41.0), 246[264-H$_2$O]$^+$ (100), 221[A/B ring]$^+$ (9.6), 203[221-H$_2$O]$^+$ (33.0), 201[246-COOH]$^+$ (93. 2).

Methylatinn of 1

A sample (20mg) was esterified when disso-

lved in MeOH and treated with CH$_2$N$_2$. The product was crystallized from MeOH as colorless needles, mp 184~6°. IR ν_{max}^{KBr}(cm^{-1}); 1725(ester).

Acetylation of 1-methylester

A sample (20mg) was acetylated with Ac$_2$O (1ml) and pyridine (0.5ml) in the usual way. The reaction product was crystallized from MeOH to give colorless plates, mp 186~8°. IR ν_{max}^{KBr} (cm^{-1}); 1730, 1235 (ester).

Formation of monobromo-γ-lactone of 1

To a solution of *1* (10mg) and NaOAc (5mg) in HOAc (1ml) was added dropwise a solution of bromine in HOAc (3%, 1ml). The reaction mixture was kept at room temp. for 3hr and then poured into H$_2$O (5ml) containing Na$_2$S$_2$O$_3$ (500mg) to discharge excess bromine. The precipitate was filtered, washed with thoroughly with H$_2$O, dried and crystallized from MeOH-H$_2$O to give a bromolactone, mp 276~80°. IR ν_{max}^{KBr}(cm^{-1}) : 1775 (γ-lactone).

LiAlH$_4$ reduction of 1-methylester

A sample (10mg) of *1*-methylester was dissolved in dry THF (30 ml) and LiAlH$_4$ (50 mg) was added slowly with stirring and then refluxed for 3 hr. The reaction mixture was filtered and subjected to CC with C$_6$H$_6$: Et$_2$O : MeOH (8 : 2 : 0.5) to give a triol (*5*), mp 248~50°. IR ν_{max}^{KBr}(cm^{-1}) : 3400(-OH), no carbonyl bond; MS(*m/z*)(rel. int.) : 458[M]$^+$ (1.0), 440[M-H$_2$O]$^+$(0.4), 427[M-CH$_2$OH]$^+$(1.3), 234[D/E ring]$^+$(10.6), 223[A/B ring]$^+$ (2.9), 203[234-CH$_2$OH]$^+$ (100).

LiAlH$_4$ reduction of hederagenin (4)-methylester

A sample (30mg) of *4*-methylester was reduced with LiAlH$_4$ (100 mg) as above to yield a triol (*5*), mp 248~50°.

Methylation of 2

A sample (20mg) was esterified with CH$_2$N$_2$

s usual. The product was crystallized from MeOH as colorless needles, mp 251~3°, IR $\nu_{max}^{KBr}(cm^{-1})$: 1720 (ester).

Acetylation of 2-methylester

A sample (20 mg) was acetylated with Ac_2O 1 ml) and pyridine (0.5 ml) in the usual way. The reaction product was crystallized from MeOH to give colorless needles, mp 174~6°. IR $\nu_{max}^{KBr}(cm^{-1})$: 1730, 1235 (ester); MS(m/z) rel. int.); 556[M]$^+$ (0.2), 496[M-HOAc]$^+$ 1.5), 437[496-OAc]$^+$ (1.2), 436[M-2×HOAc]$^+$ (0.8), 262[D/E ring]$^+$ (31.6) 293[A/B ring]$^+$ (0.2), 233[293-HOAc]$^+$ (5.4), 203[262-COOCH$_3$]$^+$ (67.5).

LiAlH$_4$ reduction of 2-methylester

A sample (10mg) of 2-methylester was reduced with LiAlH$_4$ (50 mg) as above to afford a triol (5), mp 248-50°.

Methylation of 3

A sample (5mg) was esterified when dissolved in MeOH and treated with CH_2N_2. The product was crystallized from MeOH as plates, mp 200~5°. IR ν_{max}^{KBr} (cm^{-1}) : 1725 (ester).

Acetylation of 3-methylester

A sample (5 mg) was acetylated with Ac_2O 0.5 ml) and pyridine (0.5 ml) in the usual way. The reaction product was dried under N_2 and chromatographed over SiO_2 column eluting with benzene : ether (4 : 1) to give a methylester monoacetate, mp 234~6°, and a methylester diacetate, mp 170-2°. Another sample (2 mg)

was acetylated by boiling with Ac_2O (0.5 ml) and pyridine (0.5 ml) in the usual way. The reaction product was dried under N_2 and crystallized from MeOH as needles, mp 170~2°. MS(m/z) (rel. int.); 524[M-HOAc]$^+$(10.3), 464[M-2×HOAc]$^+$ (8.6), 260[D/E ring-HOAc]$^+$ (24.3), 263[A/B ring]$^+$ (1.0), 203[263-HOAc]$^+$ (5.9), 201[260-COOCH$_3$]$^+$ (58.6).

ACKNOWLEDGEMENTS

LITERATURE CITED

1) Perry, L.M.: *Medicinal plants of East and Southeast Asia:* Attributed properties and uses. MIT, Massachusetts, p-74 (1980).

2) Woo, W.S., Shin, K.H. and Lee, C.K.: Studies on hepatotoxic substances in medicinal plants. *Arch. Pharm. Res.*, 4, 123 (1981).

3) Shin, K.H., Woo, W.S. and Lee, C.K.: Effects of *Patriniae radix* and *Melandii herba* on enzyme activities in mice. *Kor. J. Pharmacogn.*, 16, 1 (1985).

4) Budzikiewicz, H., Wilson, J.M., and Djerassi, C.: Mass spectrometry in structural and stereochemical problems XXXII. Pentacyclic triterpenes. *J. Am. Chem. Soc.*, 85, 3688 (1963).

5) Shamma, M., Glick, R.E. and Mumma, R.O.: The nuclear magnetic resonance spectra of pentacyclic triterpenes. *J. Org. Chem.*, 27, 4512 (1962).