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Chemical Constituents from the Aerial Part of *Rosa transmorrisonensis*

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The aerial part of *Rosa transmorrisonensis* Hayata contains chemical constituents of long-chain alkanes, linolenic acid, squalene, catechin, sitosterol, sitosteryl-β-D-glucoside, 3,4,5-trimethoxyphenyl-β-D-glucoside, 9-glucosyl-4,7*E*-megastigmadien-3-one, oleanolic acid and eight ursolic acid derivatives. Among them, the new compound **14a** was determined to be 2,3,4,6-tetraacetyl-(19α-hydroxy-2α,3α,24-triacetoxyurs-12-en-28-oyl)-β-D-glucopyranoside by spectral methods.

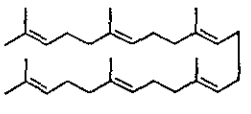
INTRODUCTION

Rosa transmorrisonensis Hayata (高山薔薇), Rosaceae, is a shrub common in thickets at high altitudes (1800-3200 m) in Taiwan. The chemical constituents of this plant have not been investigated; some bioactive compounds have been found in other species of Rosa genus.[1] We have previously investigated the chemical constituents of *Rosa taiwanensis* Nakai (小金櫻) and *Rosa laevigata* Michx. (大金櫻).[2] The former plant has components of long-chain alcohols, sitosterol, campesterol, phytol, euscaphic acid, lupeol, betulinic acid, tormentic acid and its glucoside. The latter plant contains compounds of Henze's ketol, diethyl malate, loliolide, *p*-coumaric acid, 6,7-dimethoxycoumarin, γ-lactones, flavonoids, steroids and triterpenes of ursolic-, euscaphic- and oleanolic-types. We here report the chemical constituents isolated from the acetone-soluble fraction of the aerial part of *R. transmorrisonensis*.

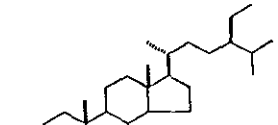
RESULTS AND DISCUSSION

The acetone extract of the air-dried aerial part of *R. transmorrisonensis* was filtered through a charcoal column, the filtrate was concentrated and chromatographed to give fractions A-E in ascending order of polarity. Long-chain alkanes, squalene (**1**) and sitosterol (**2**) were isolated from the least polar fraction A. Fractions B-D of medium polarity were subjected to methylation with diazomethane to give catechin (**3**) and methyl esters **4m-10m**. Acetylation of the most polar fraction E afforded the glucoside polyacetates **11a-16a**. However, the natural products are expected to exist as the parent compounds **4-16** because the ^1H NMR spectra of the samples without chemical modification showed no acetyl or methoxycarbonyl signal. The known compounds **1-3** were readily recognized by comparison of physical and spectral properties (mp, [α], MS, IR, ^1H and ^{13}C NMR) with those of authentic samples.[3-7] (-)-Catechin is also a component of the aerial part of *R. laevigata*.[2]

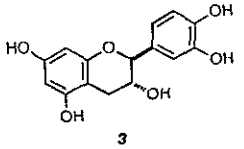
Ursolic acid **4** *is the predominant component*, comprising about one percent of the weight of the dried plant material. The methyl ester **4m** obtained by crystallization from MeOH had mp 110-112 °C (lit. 111-114 °C) and $[\alpha]_D^{25}$ +66.1° ($CHCl_3$, c 2.5).[8,9] Methyl oleanolate (**5m**), mp 199-201 °C (lit.[8,9] 200-203 °C), was also isolated. Other known ursolic acid derivatives, methyl 2α,3β-dihydroxyurs-12-en-28-oate **6m**,[8-12] methyl 2α,3β,19α-trihydroxyurs-12-en-28-oate **7m** (methyl tormentate)[12-14] and methyl 2α,3α,19α-trihydroxyurs-12-en-28-oate **8m**,[10,11] were characterized. The common features in the ^1H NMR spectra of these compounds include a CO_2Me group and seven methyl groups. The stereochemisty of **8m** was established by irradiation of the Me-4β resonance at δ 0.83 to cause 7.8 and 6.9 percent



1



2 R = H
11 R = Glu
11a R = Glu(OAc)$_4$



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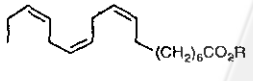
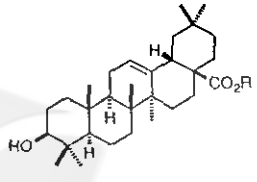


10 R = H
10m R = Me



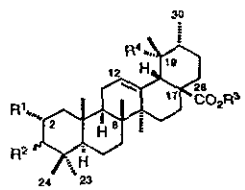
5 R = H
5m R = Me

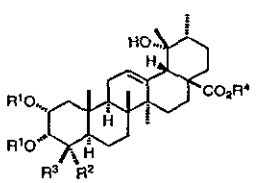
Dedicated to Professor Fa-Ching Chen (陳發清) on the occasion of his eightieth birthday.

nuclear Overhauser enhancements of H-2β at δ 3.97 and H-3β at δ 3.40 respectively. The H-2 and H-3 in **6m** and **7m** were axially oriented as indicated by a large coupling constant 9.4 Hz ($J_{2\beta,3\alpha}$), whereas the corresponding coupling constant $J_{2\beta,3\beta}$ was small (3.5 Hz) in **8m**. The mass spectrum of methyl ester **9m** showed a parent signal at m/z 518 corresponding to the molecular formula $C_{31}H_{50}O_6$. The ^{1}H NMR spectrum showed signals at δ 0.64 (*s*), 0.69 (*s*), 0.90 (*s*), 0.91 (*d*), 1.18 (*s*) and 1.24 (*s*) for six methyl groups, and a signal at δ 3.43-3.85 for four protons geminal to hydroxyl groups. We inferred that **9m** containing a CH_2OH group is also a ursolic acid derivative, 2α,3α,19α,23-tetrahydroxyurs-12-en-28-oate. The compound had mp 136-138 °C and $[\alpha]_D$ +26.9° near reported values.[14-16] As the CH_2OH group is equatorial, there is no shielding effect on the Me-10 group by comparison of the chemical shift in **9m** (δ 0.90) with that of **8m** (δ 0.93).

An oil **10m** showed M^+ at m/z 292, vinyl proton signals at o 5.28-5.38 and an IR absorption at 1737 cm^{-1} attributable to an ester group. By comparison of the spectra with those of authentic sample, **10m** was determined to be methyl 9,12,15-octadecatrienoate, i.e. linolenic acid methyl ester.[3]

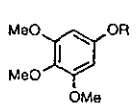
The peracetylated compound **11a**, mp 165-167 °C, was determined to be the known compound 1-sitosteryl-2,3,4,6-tetraacetyl-β-D-glucopyranoside.[10,17] The carbon of the glucose moiety showed characteristic signals at δ 99.6 (C-1′), 71.6 (C-2′), 71.5 (C-3′), 68.5 (C-4′), 72.9 (C-5′) and 62.1 (C-6′). Spectral analysis of **12a** revealed that it was a carbohydrate derivative of the ursolic acid **8**, 2,3,4,6-tetraacetyl-(2α,3α,19α-trihydroxyurs-12-en-28-oyl)-β-D-glucopyranoside.[14,16] The assignment of proton signals in **12a** was established by an H-H COSY spectrum.



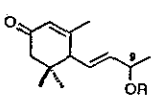


	R^1	R^2	R^3	R^4
4	H	β-OH	H	H
4m	H	β-OH	Me	H
6	OH	β-OH	H	H
6m	OH	β-OH	Me	H
7	OH	β-OH	H	OH
7m	OH	β-OH	Me	OH
8	OH	α-OH	H	OH
8m	OH	α-OH	Me	OH
12	OH	α-OH	Glu	OH
12a	OH	α-OH	Glu(OAc)$_4$	OH

	R^1	R^2	R^3	R^4
9	H	CH_2OH	Me	H
9m	H	CH_2OH	Me	Me
13	H	Me	CH_2OH	H
13a	Ac	Me	CH_2OAc	H
14	H	Me	CH_2OH	Glu
14a	Ac	Me	CH_2OAc	Glu(OAc)$_4$



15 R = Glu
15a R = Glu(OAc)$_4$



16 R = Glu
16a R = Glu(OAc)$_4$

In summary, the aerial part of *R. transmorrisonensis* contains a series of ursolic acid derivatives. Compounds **4-8** and **12** are common constituents in *R. transmorrisonensis* and *R. laevigata*. Only tormentic acid **7** is found in *R. taiwanensis*. As a related compound 2α-hydroxyurs-12-en-28-oic acid exhibits activity against human colon HCT-8 tumor cell,[13] further pharmacological tests of these compounds occurring in *R. transmorrisonensis* may provide valuable information.

EXPERIMENTAL SECTION

Instruments

Yanagimoto (or MP-500D) micro melting point apparatus, JASCO Dip-180 digital polarimeter, Finnigan TSQ-46c mass spectrometer, Perkin-Elmer 983G infrared spectrophotometer, Bruker AM-300 WB (or AC 200) nuclear magnetic resonance spectrometer, and Waters M-45 high performance liquid chromatograph were used.

Plant Material

The aerial parts of *Rosa transmorrisonensis* Hayata (3.1 kg) were collected from Tayuling (大禹嶺) in July 1988. A specimen of this plant has been deposited in our laboratory. The aerial parts without fruit were exhaustively

extracted with acetone (15 L × 3). These extracts were passed through a short column of activated charcoal. The filtrate was concentrated, the residue (58 g) was coated with silica gel (75 g) and subjected to chromatography on a silica gel (950 g) column by elution with gradients of ethyl acetate and hexane. The appropriate portions were combined to give five fractions A-E in ascending order of polarity. The least polar fraction A was further separated by flash chromatography to give long-chain alkanes, squalene (**1**) and sitosterol (**2**). Fraction B was subjected to methylation with diazomethane and separated by HPLC to give catechin (**3**) and the methyl esters **4m-8m**. According to a similar procedure, the fractions C and D were methylated and separated to give **4m-9m** and **10m**, respectively. Acetylation of the most polar fraction E with Ac_2O in pyridine, followed by separation on a silica gel column, afforded the polyacetates **11a-16a**. The isolated weights were **1** (60 mg), **2** (400 mg), **3** (385 mg), **4m** (540 mg), **5m** (375 mg), **6m** (284 mg), **7m** (187 mg), **8m** (250 mg), **9m** (18 mg), **10m** (23 mg), **11a** (32 mg), **12a** (65 mg), **13a** (25 mg), **14a** (15 mg), **15a** (20 mg) and **16a** (15 mg).

The data of the new compound **14a** and additional data of known compounds are listed as follows.

Squalene (1)[3]

Oil; R_f 0.75 (EtOAc/hexane, 1:10); ^{13}C NMR ($CDCl_3$) δ 15.98 (C-1, 24), 16.0 (C-25, 30), 17.7 (C-27, 28), 25.7 (C-26, 29), 26.7 (C-12, 13), 26.8 (C-9, 16), 28.3 (C-4, 21), 39.8 (C-5, 20), 124.3 (C-11, 14), 124.31 (C-7, 18), 124.4 (C-3, 22), 131.2 (C-2, 23), 134.9 (C-10, 15), 135.1 (C-6, 19).

Sitosterol (2)[4,5]

Needle crystals; mp 136-138 °C (*lit.*[4] 137-138 °C); R_f 0.25 (EtOAc/hexane, 1:4).

Catechin (3)[6,7]

Mp 174-176 °C (lit.[6] 175-178 °C); $[\alpha]_D^{25}$ -3.9° (Me_2CO, c 7.1), lit.[6] -4.1° (Me_2CO, c 0.41); R_f 0.27 (EtOAc/hexane, 3:7).

Methyl Ursolate (4m)[8,9]

Needle crystals from MeOH; mp 110-112 °C (lit.[8] 111-114 °C); $[\alpha]_D^{25}$ +66.1° ($CHCl_3$, c 2.5), lit.[8] +66.5° ($CHCl_3$, c 1.02); R_f 0.30 (EtOAc/hexane, 2:3).

Methyl Oleanolate (5m)[8,9]

Needle crystals from MeOH; mp 199-201 °C (lit.[8] 200-203 °C); $[\alpha]_D^{25}$ +60.9° ($CHCl_3$, c 2.2), lit.[8] +66.7° ($CHCl_3$, c 0.87); R_f 0.35 (EtOAc/$CHCl_3$, 1:2).

Methyl 2α,3β-Dihydroxyurs-12-en-28-oate (6m)[8-12]

Needle crystals from MeOH; mp 203-205 °C (lit.[8] 204-207 °C); $[\alpha]_D^{25}$ +40.2° ($CHCl_3$, c 5.2), lit.[10] +39.8° ($CHCl_3$, c 8.6); R_f 0.35 (EtOAc/$CHCl_3$, 1:2).

Methyl 2α,3β,19α-Trihydroxyurs-12-en-28-oate (7m)[12-14]

Needle crystals from MeOH; mp 145-147 °C (lit.[12] 145-150 °C); $[\alpha]_D^{25}$ +31.2° ($CHCl_3$, c 4.3), lit.[12] +34.0° ($CHCl_3$, c 0.2); R_f 0.42 (EtOAc/$CHCl_3$, 1:3).

Methyl 2α,3α,19α-Trihydroxyurs-12-en-28-oate (8m)[10,11]

Needle crystals from MeOH; mp 124-126 °C (lit.[10] 125-130 °C); $[\alpha]_D^{25}$ +20.2° ($CHCl_3$, c 8.1), lit.[11] +23.8° ($CHCl_3$, c 8.9); R_f 0.45 (EtOAc/$CHCl_3$, 2:5).

Methyl 2α,3α,19α,23-Tetrahydroxyurs-12-en-28-oate (9m)[14-16]

Needle crystals from MeOH; mp 136-138 °C (lit.[15] 137-139 °C); $[\alpha]_D^{25}$ +26.9° ($CHCl_3$, c 4.5), lit.[16] +31.9° ($CHCl_3$, c 1.65); R_f 0.26 (EtOAc/CH_2Cl_2, 2:1).

Methyl 9,12,15-Octadecatrienoate (10m)[3]

Oil; R_f 0.62 (EtOAc/$CHCl_3$, 1:3).

1-Sitosteryl-2,3,4,6-tetraacetyl-β-D-glucopyranoside (11a)[10,17]

Needle crystals from MeOH; mp 165-167 °C (lit.[10] 169-170 °C); $[\alpha]_D^{25}$ -28.1° ($CHCl_3$, c 1.1), lit.[10] -29.1° ($CHCl_3$, c 2.1); R_f 0.20 (EtOAc/$CHCl_3$, 1:6).

2,3,4,6-Tetraacetyl-(2α,3α,19α-trihydroxyurs-12-en-28-oyl)-β-D-glucopyranoside (12a)[14,16]

Plate crystals from MeOH; mp 157-161 °C, $[\alpha]_D^{25}$ +31.6° ($CHCl_3$, c 1.9); R_f 0.15 (EtOAc/$CHCl_3$, 1:6).

19α-Hydroxy-2α,3α,24-triacetoxyurs-12-en-28-oic Acid (13a)[14,18]

Plate crystals from MeOH; mp 134-138 °; $[\alpha]_D^{25}$ +30.4° ($CHCl_3$, c 4.6); R_f 0.60 (EtOAc/$CHCl_3$, 1:6).

2,3,4,6-Tetraacetyl-(19α-hydroxy-2α,3α,24-triacetoxyurs-12-en-28-oyl)-β-D-glucopyranoside (14a)

Needle crystals from MeOH; mp 169-171 °C; $[\alpha]_D^{25}$ +26.5° ($CHCl_3$, c 4.9); R_f 0.45 (EtOAc/$CHCl_3$, 1:3); IR (KBr) 3550, 2934, 1741, 1430, 1365, 1230, 1167, 1036, 910 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.68 (*s*, Me), 0.90 (*d*, J = 6.2 Hz, H-30), 1.04 (*s*, Me), 1.07 (*s*, Me), 1.16 (*s*, Me), 1.27 (*s*, Me), 2.49 (*br s*, H-18), 3.80-3.88 (*m*, H-5′), 3.71 (*d*, J = 10.5 Hz, H-24), 4.01 (*dd*, J = 12.3, 2.5 Hz, H-6′), 4.04 (*d*, J = 10.5 Hz, H-24), 4.23 (*dd*, J = 12.3, 4.4 Hz, H-6′), 5.08 (*t*, J = 9.1 Hz, H-4′), 5.10 (*dd*, J = 9.1, 7.8 Hz, H-2′), 5.17 (*m*, H-2), 5.20 (*t*, J = 9.1 Hz, H-3′), 5.34 (*br s*, H-12), 5.47 (*br s*, H-3), 5.49 (*d*, J = 7.8 Hz, H-1′); ^{13}C NMR ($CDCl_3$) δ 16.0 (C-25), 16.3 (C-23), 16.9 (C-30), 17.2 (C-26), 18.4 (C-6), 20.5, 20.6, 20.7, 20.8, 20.9, 21.0, 23.5 (C-11), 24.1 (C-27), 25.2 (C-16), 25.7 (C-21), 27.3 (C-29), 28.2 (C-15), 32.4 (C-7), 36.5 (C-22), 38.3 (C-8), 38.5 (C-1), 39.8 (C-10), 40.9 (C-14), 41.0 (C-4), 41.3 (C-20), 46.8 (C-9), 47.0 (C-5), 47.9 (C-17), 52.9 (C-

18), 61.5 (C-6′), 67.8 (C-2), 68.0 (C-4′), 69.8 (C-2′, C-24), 71.9 (C-3), 72.4 (C-3′), 72.8 (C-5′), 73.0 (C-19), 91.7 (C-1′), 128.8 (C-12), 137.5 (C-13), 168.8, 169.4, 170.1, 170.4, 170.5, 171.3, 175.7 (C-28); $C_{50}H_{72}O_{18}$, FABMS m/z 983 $(M+Na)^+$, 917 (M^+-CH_3CO), 901 $(M^+-CH_3CO_2)$, 512, 331.

2,3,4,6-Tetraacetyl-3,4,5-trimethoxyphenyl-β-D-glucoside (15a)[19,20]

Oil; R_f 0.32 (EtOAc/$CHCl_3$, 3:7).

2,3,4,6-Tetraacetyl-(4,7*E*-megastigmadien-3-one-9-yl)-β-D-glucoside (16a)[21]

Oil; $[\alpha]_D^{30}$ +47.0° (MeOH, c 6.6); R_f 0.25 (EtOAc/$CHCl_3$, 3:7).

ACKNOWLEDGMENT

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Key Words

Rosa transmorrisonensis; Rosaceae; Aerial part; Ursolic acid derivatives.

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