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TRITERPENOIDS FROM *ENKIANTHUS CAMPANULATUS*

JINSAKU SAKAKIBARA, TOYO KAIYA and HARUYO FUKUDA

Faculty of Pharmaceutical Sciences, Nagoya City University, Tanabe-dori, Mizuho-ku, Nagoya 467, Japan

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Key Word Index—*Enkianthus campanulatus*; *Ericaceae*; triterpenes; 3-oxo-19,23,24-trihydroxyurs-12-en-28-oic acid; 3β,6β,19,23-tetrahydroxyurs-12-en-28-oic acid; 3β,6β,23-trihydroxyurs-12-en-28-oic acid; sumaresinolic acid; rotundic acid.

Abstract—From the leaves of *Enkianthus campanulatus* were isolated three new triterpenes, 3-oxo-19,23,24-trihydroxyurs-12-en-28-oic acid, 3β,6β,19,23-tetrahydroxyurs-12-en-28-oic acid and 3β,6β,23-trihydroxyurs-12-en-28-oic acid.

INTRODUCTION

Enkianthus campanulatus Nichols is a wild shrub distributed in the eastern part of Japan and used as a decorative plant. Several phenolic compounds, e.g. lyoniside, vitexin, daphnin and skimmin [1] have been isolated from this plant. However, triterpenoid components have never been investigated. As a part of our study on the constituents of plants of the Ericaceae [2], we wish to report here the isolation and structural elucidation of three new triterpenes along with several known compounds from the title plant.

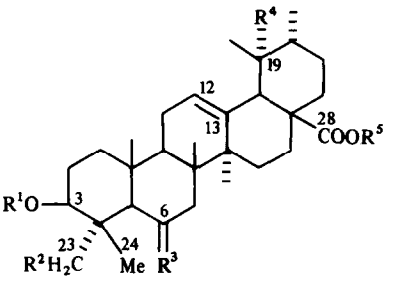
RESULTS AND DISCUSSION

The leaves of the plant were extracted with benzene and methanol, successively. The benzene extract was filtered to remove precipitated ursolic acid (**1**). The filtrate was adsorbed onto silica gel and washed with hexane. The hexane insoluble fraction was chromatographed over a silica gel column, and sitosterol (**2**), sumaresinolic acid (3β,6β-dihydroxyolean 12-en-28-oic acid) (**3**) [3] and sitosteryl β-D-glucoside (**4**) were isolated.

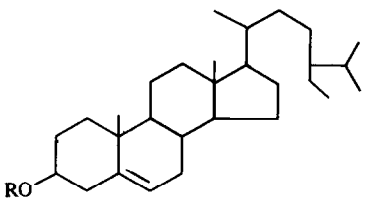
The methanol extract was diluted with water and extracted with chloroform. The chloroform-soluble fraction was chromatographed over a silica gel column and afforded a mixture of triterpenes, which was separated into four compounds, **5**, **6**, **7** and **8**, by repeated column chromatography and/or prep. TLC. Compound **5** was identified as rotundic acid (3β,19,23-trihydroxyurs-12-en-28-oic acid) [4] by spectroscopic means.

The molecular formulas of **6**, **7** and **8** were revealed to be $C_{30}H_{46}O_6$, $C_{30}H_{48}O_6$ and $C_{30}H_{48}O_5$, respectively, by high resolution mass spectrometry of their derivatives. They gave monomethyl esters on methylation with diazomethane. Their ^{13}C NMR spectra showed a signal at δ137–138 for C-13, indicating that they should have an ursene skeleton [5]. The ^1H NMR spectra of acetates of **6** and **7** (**6a** and **7a**) showed a one proton singlet at δ2.5 for H-18, and the mass spectra of their methyl esters (**6b** and **7b**) exhibited peaks at *m/z* 278, 260, 201 and 179, suggesting that they had a 28-carbomethoxy-19-hydroxyurs-12-ene structure [2].

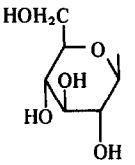
On acetylation **6** gave a diacetate (**6a**), whose ^1H NMR and ^{13}C NMR spectra showed the presence of two primary acetoxyl groups [δ3.94 and 4.69 (each 1H, *d*, *J* = 12 Hz), 4.21 and 4.45 (each 1H, *d*, *J* = 12 Hz)] and one carbonyl group (δ209.9). These groups must be located on rings A and/or B as shown by the mass spectrum.

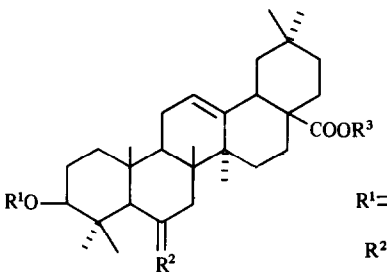


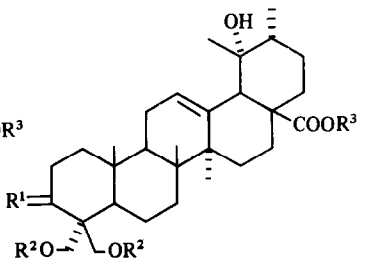
	R^1	R^2	R^3	R^4	R^5
1	H	H	H_2	H	H
1a	Ac	H	H_2	H	Me
5	H	OH	H_2	OH	H
5a	Ac	OAc	H_2	OH	H
5b	H	OH	H_2	OH	Me
5c	Ac	OAc	H_2	OH	Me
7	H	OH	HO, H	OH	H
7a	Ac	OAc	HO, H	OH	H
7b	H	OH	HO, H	OH	Me
7c	Ac	OAc	HO, H	OH	Me
8	H	OH	HO, H	H	H
8a	Ac	OAc	HO, H	H	H
8b	H	OAc	HO, H	H	Me
10	Ac	H	HO, H	H	Me
10a	Ac	H	O	H	Me



	R
2	H







	R^1	R^2	R^3
3	H	HO, H	H
3a	Ac	HO, H	Me
3b	Ac	O	Me
11	Ac	H_2	Me

	R^1	R^2	R^3
6	O	H	H
6a	O	Ac	H
6b	O	H	Me
9	AcO, H	Ac	Me

Takahashi and his coworkers have reported the assignments of the ^{13}C NMR spectra of some 19-hydroxyurs-12-en-28-oic acid derivatives [6]. Comparing the ^{13}C NMR spectrum of **6a** with that of methyl triacetylclethrate (methyl 3β,23,24-triacetoxyurs-12-en-28-oate, **9**), only the C-2 and C-4 signals shifted downfield by more than 10 ppm (Table 1). Thus, the position of the carbonyl group could be attributed to C-3 and the two primary hydroxyl groups to C-23 and C-24. Consequently the structure of **6** was established as 3-oxo-19,23,24-trihydroxyurs-12-en-28-oic acid.

Compound **7b** gave a diacetate (**7c**), whose ^1H NMR and ^{13}C NMR spectra were very similar to those of methyl diacetylrotundate (**5c**) (Tables 1 and 2), and showed the presence of primary and secondary acetoxyl groups, and a secondary hydroxyl group in rings A and/or B. Comparing the ^{13}C NMR spectra of **5c** and **7c**, the signal due to C-6 (δ18.1) of **5c** disappeared, instead a doublet peak at δ68.1 appeared in the spectrum of **7c**, and the C-7 of **5c** (δ32.5) shifted downfield to δ41.0. Other peaks remained almost unchanged. Therefore the structure of **7** was deduced to be 3β,6,19,23-tetrahydroxyurs-12-en-28-oic acid. The ^1H NMR spectrum of **7c** showed a peak at δ4.38 (1H, *br s*, $W_{1/2}$ = 8 Hz) due to H-6, which was in good agreement to that of methyl acetyl-6β-hydroxyursolate [δ4.50 ($W_{1/2}$ = 8 Hz)], previously isolated from *E. cernuus* [7]. Moreover the calculated values of tertiary methyl signals for 6β-hydroxyrotundic acid according to the method reported by Cheung *et al.* [8] (Table 3) agreed well with the experimental ones. In consequence the structure of **7** was concluded to be 3β,6β,19,23-tetrahydroxyurs-12-en-28-oic acid.

Two significant peaks at *m/z* 248 and 203 in the mass spectrum of **8** gave definite evidence for the presence of the urs-12-ene skeleton having a carboxyl and no hydroxyl groups on rings D and/or E [7]. The ^1H NMR and ^{13}C NMR spectra of its methyl ester diacetate (**8b**) closely resembled those of **7c** (Tables 1 and 2) except that the former lacked a peak due to H-18. Thus the structure of **8** was deduced to be 3β,6β,23-trihydroxyurs-12-en-28-oic acid.

EXPERIMENTAL

All mps are uncorr. The IR spectra were recorded on KBr discs. The ^1H NMR spectra were run at 200 and 100 MHz, and the

Table 1. ^{13}C NMR chemical shifts (δ, $CDCl_3$)

	3b	5c	6a	7c	8b	9
C-1	38.6	37.9	38.5	39.9	39.9	33.2
2	23.3	23.0	35.2	23.1	23.6	22.4
3	80.0	74.6	209.9	74.6	74.5	70.3
4	46.2	40.6	54.5	41.6	42.4	43.0
5	65.3	47.9	49.8	48.7	48.5	47.9
6	211.4	18.1	19.4	68.4	68.1	19.4
7	50.5	32.5	32.5	41.0	41.0	32.8
8	38.8	40.0	39.9	39.0	38.6	39.9
9	47.9	47.3	46.7	47.5	47.8	47.3
10	36.6	36.8	36.4	36.3	36.2	36.7
11	22.9	23.7	23.9	23.6	23.2	23.7
12	121.9	129.0	128.6	129.2	125.8	128.7
13	143.6	138.2	138.2	137.3	137.2	138.0
14	42.8	41.2	41.2	41.3	41.3	41.1
15	27.5	28.3	28.2	28.1	28.0	28.2
16	23.8	25.5	25.3*	25.4	24.2	25.5*
17	46.6	47.9	47.8	47.9	48.1	47.9
18	41.3	53.3	52.9	53.2	52.9	53.3
19	45.6	73.2	73.1	73.2	39.1	73.1
20	30.7	41.2	41.1	41.2	40.1	41.1
21	33.8	26.1	25.9*	26.0	30.7	26.0*
22	32.2	37.4	37.4	37.4	36.6	37.4
23	27.6	65.5	63.7†	65.6	65.6	64.3
24	17.2	13.1	63.2†	17.8	18.4	67.2
25	16.4	15.8	15.2	14.5	14.5	15.2
26	16.4	16.7	16.6‡	17.2	17.4	16.5†
27	26.3	24.4	24.3	24.4	23.2	24.4
28	177.9	178.3	184.0	178.2	177.9	178.2
29	33.1	27.5	27.4	27.4	17.0	27.4
30	23.6	16.1	16.1‡	16.1	21.2	16.1†
OMe	51.6	51.5		51.6	51.5	51.5
		20.9		20.9	20.9	20.9
COMe	21.2	21.2	21.2	21.2	21.2	20.9
		170.6		170.7	170.7	169.9
COMe	170.9	170.9	170.5	170.9	170.9	170.5

*,†,‡Values in any vertical column may be reversed.

^{13}C NMR spectra at 50 and 25 MHz with TMS as internal standard. Mass spectra (70 eV) were taken with a direct inlet.

Plant material. Leaves of *E. campanulatus* Nichols were collected in Tottori prefecture, Japan, in 1981 by Chizu Agricultural Cooperative of Tottori.

Extraction and isolation. The powdered dried leaves (7.49 kg) were extracted under reflux with C_6H_6 and MeOH, successively. Ppts were removed from the C_6H_6 extract, to which was added silica gel and the solvent evaporated. The adsorbent was extracted with hexane and filtered with suction. The filtrate was applied to a silica gel column and eluted with a mixture of C_6H_6–EtOAc. Sitosterol (**2**) was obtained from C_6H_6–EtOAc (9:1) eluate, compound **3** from the C_6H_6–EtOAc (7:3) eluate, and sitosteryl β-D-glucoside (**4**) from the EtOAc eluate.

The MeOH extract was concd, diluted with H_2O and extracted with $CHCl_3$ at room temp. The $CHCl_3$ soluble portion was chromatographed on a silica gel column with a mixture of $CHCl_3$–MeOH. From the $CHCl_3$–MeOH (99:1) eluate was obtained a mixture of triterpenes, which was separated into four compounds (**5**, **6**, **7** and **8**) by repeated column chromatography and/or prep. TLC (developing solvent: C_6H_6–EtOAc). Acetylation and methylation of these compounds were carried out in the usual manner with Ac_2O and pyridine, and CH_2N_2 respectively, at room temp.

Ursolic acid (**1**). The ppts from the C_6H_6 extract were collected, treated with activated charcoal and recrystallized from EtOH, mp 269–273°. Identical with the authentic sample.

Sitosterol (**2**). Purified as the acetate followed by alkaline hydrolysis. Recrystallized from MeOH, mp 141.5°. Identical with the authentic sample.

Sumaresinolic acid (**3**). Purified as the methyl ester acetate (**3a**). IR ν_{max}^{KBr} cm^{-1}: 3480, 1720, 1240. ^1H NMR ($CDCl_3$): δ0.90, 0.93, 0.95, 1.03, 1.10, 1.24, 1.32 (each 3H, *s*), 2.02 (3H, *s*), 3.60 (3H, *s*), 4.40 (1H, *m*), 4.52 (1H, *br s*, $W_{1/2}$ = 8 Hz), 5.32 (1H, *m*). These data were in good agreement with those in the literature [3].

Methyl 3-acetyl-6-didehydrosumaresinolate (**3b**). Compound **3a** was treated with Jones reagent as in the case of 6-hydroxyursolic acid [7]. Mp 296–304° (MeOH). IR ν_{max}^{KBr} cm^{-1}: 1722, 1703, 1240. ^1H NMR ($CDCl_3$): δ0.88 (3H, *s*), 0.93 (12H, *s*), 1.24, 1.28 (each 3H, *s*), 2.03 (3H, *s*), 2.20 (1H, *s*), 1.86, 2.44 (each 1H, *d*, *J* = 12 Hz), 2.86 (1H, AB*q*, *J* = 3 and 16 Hz), 4.36 (1H, *t*, *J* = 7 Hz), 5.26 (1H, *m*). ^{13}C NMR ($CDCl_3$): see Table 1. The differences of the chemical shifts of C-5, C-6 and C-7 between methyl acetyloleanolate (**11**) [9] and **3b** (Table 4) are very similar to those observed between methyl acetylursolate (**1a**) [9] and methyl acetyl-6-oxoursolate (**10a**) [7]. From these findings **3** was concluded to be sumaresinolic acid.

Sitosteryl β-D-glucoside (**4**). Recrystallized from $CHCl_3$–MeOH, mp 288–289°. Identical with the authentic specimen.

Rotundic acid (**5**). Mp 269–274° (MeOH). ^1H NMR (C_5D_5N): δ1.00, 1.04, 1.08, 1.42, 1.64 (each 3H, *s*), 3.00 (1H, *s*), 3.66, 4.10 (each 1H, *d*, *J* = 11 Hz), 5.59 (1H, *m*).

Diacetylrotundic acid (**5a**). Mp 168–177° (MeOH). ^1H NMR: see Table 2.

Methyl rotundate (**5b**). Mp 252–255°. ^1H NMR ($CDCl_3$): δ0.69, 0.88, 0.96, 1.21, 1.26 (each 3H, *s*), 2.60 (1H, *s*), 3.40, 3.70 (each 1H, *d*, *J* = 12 Hz), 3.60 (3H, *s*), 3.60 (1H, *m*), 5.36 (1H, *m*). The IR spectrum was identical with that of the authentic sample.

Methyl diacetylrotundate (**5c**). ^1H NMR: see Table 2. ^{13}C NMR: see Table 1. The IR spectrum was identical with that of the authentic sample. From these findings **5** was identified as rotundic acid [4].

3-Oxo-19,23,24-trihydroxyurs-12-en-28-oic acid (**6**). Amorphous powder, $[\alpha]_D^{26}$ +19.9° (*c* 2.33, MeOH). High resolution MS *m/z*: 502.3266 (M^+, calc. for $C_{30}H_{46}O_6$, 502.3294). ^1H NMR (C_5D_5N): δ0.98, 1.09, 1.45, 1.66 (each 3H, *s*), 3.00 (1H, *s*), 3.93 (1H, *d*, *J* = 11 Hz), 4.28, 4.51 (each 1H, *d*, *J* = 11 Hz), 4.08 (1H, *m*), 4.76 (1H, *d*, *J* = 11), 5.60 (1H, *m*).

23,24-Diacetoxy-19-hydroxy-3-oxo-urs-12-en-28-oic acid (**6a**). IR ν_{max}^{KBr} cm^{-1}: 3400, 1732, 1715, 1695, 1225. ^1H NMR ($CDCl_3$): δ0.78, 0.98, 1.20, 1.26 (each 3H, *s*), 1.96, 2.00 (each 3H, *s*), 2.54 (1H, *s*), 3.94 (1H, *d*, *J* = 12 Hz), 4.21, 4.45 (each 1H, *d*, *J* = 12 Hz), 4.69 (1H, *d*, *J* = 12 Hz), 5.24 (1H, *m*, *J* = 12 Hz). ^{13}C NMR ($CDCl_3$): see Table 1.

Methyl 3-oxo-19,23,24-trihydroxyurs-12-en-28-oate (**6b**). MS *m/z*: 516 (M^+, $C_{31}H_{48}O_6$), 278, 260, 201, 179.

3,23-Diacetoxy-6,19-dihydroxyurs-12-en-28-oic acid (**7a**). ^1H NMR: see Table 2.

Methyl 3,6,19,23-tetrahydroxyurs-12-en-28-oate (**7b**). MS *m/z*: 518 (M^+, $C_{31}H_{50}O_6$), 278, 260, 201, 179, 146.

Methyl 3,23-diacetoxy-6,19-dihydroxyurs-12-en-28-oate (**7c**). Amorphous powder. IR ν_{max}^{KBr} cm^{-1}: 3460, 1730, 1715, 1240. High resolution MS *m/z*: 602.3831 (M^+, calc. for $C_{35}H_{54}O_8$, 602.3818). ^1H NMR: see Table 2. ^{13}C NMR: see Table 1. MS *m/z* 602 $[M]^+$, 278, 179, 146.

3,6,23-Trihydroxyurs-12-en-28-oic acid (**8**). Amorphous

Table 2. ^{1}H NMR chemical shifts (δ, $CDCl_3$)

	H-3 (*t*)	H-6 (*br s*)*	H-12 (*m*)	H-18 (*s*)	H-23 (*d*)	H-24 (*s*)	H-25 (*s*)	H-26 (*s*)	H-27 (*s*)	H-29 (*s*)	COMe (*s*)	OMe (*s*)
5a	4.80 (J = 8)		5.36	2.55	3.71 3.90 (J = 12) 3.72	0.84	1.00	0.75	1.22	1.26	2.03 2.07	
5c	4.79 (J = 8)		5.37	2.60	3.90 (J = 11) 3.82	0.85	0.98	0.70	1.22	1.27	2.02 2.06	3.61
7a	4.72 (J = 8)	4.35 ($W_{1/2}$ = 9)	5.38	2.55	3.99 (J = 12) 3.86	1.23	1.35	1.04	1.23	1.23	2.03 (6H)	
7c	4.76 (J = 8)	4.38 ($W_{1/2}$ = 8)	5.42	2.64	4.00 (J = 12) 3.83	1.23	1.36	0.97	1.23	1.23	2.04 2.05	3.61
8a	4.74 (J = 7)	4.34 ($W_{1/2}$ = 8)	5.30		4.00 (J = 12) 3.80	1.27	1.36	1.07	1.23		2.03 (6H)	
8b	4.72 (J = 7)	4.33 ($W_{1/2}$ = 8)	5.30		3.90 (J = 12)	1.24	1.36	1.03	1.24		2.02 (6H)	3.60

Table 3. Calculated ^{1}H NMR methyl signals for **7a** and **7c** (based on **5a** and **5c**)

	H-24	H-25	H-26	H-27
Increments for 6β-OH	+0.39	+0.38	+0.31	−0.05
7a	1.23	1.38	1.06	1.17
7c	1.24	1.36	1.01	1.17

Table 4. Comparison of the ^{13}C NMR spectra of compounds **3b** and **11** and of **10a** and **1a**

	3b	**11**	Δ	**10a**	**1a**	Δ
C-5	65.3	55.2	10.1	64.9	55.3	9.6
C-6	211.4	18.2	193.2	211.4	18.1	193.3
C-7	50.5	32.6	17.9	50.6	32.8	17.8

powder, $[\alpha]_D^{26}$ +41.2° (*c* 1.70, MeOH). High resolution MS *m/z*: 488.3526 (M^+, calc. for $C_{30}H_{48}O_5$, 488.3502). MS: *m/z* 488 $[M]^+$, 470, 452, 248, 222, 203.

3,23-Diacetoxy-6-hydroxyurs-12-en-28-oic acid (**8a**). IR ν_{max}^{KBr} cm^{-1}: 3480, 1720, 1695, 1235. ^{1}H NMR: see Table 2.

Methyl 3,23-diacetoxy-6-hydroxyurs-12-en-28-oate (**8b**). ^{1}H NMR: see Table 2. ^{13}C NMR: see Table 1.

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