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TWO NOVEL HEXACYCLIC TRITERPENOIDS FROM *PRUNELLA VULGARIS**

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Key Word Index—*Prunella vulgaris*; Labiatae; triterpenoids; (12*R*, 13*S*)-2α,3α,24-trihydroxy-12,13-cyclo-taraxer-14-en-28-oic acid; (13*S*,14*R*)-2α,3α,24-trihydroxy-13,14-cyclo-olean-11-en-28-oic acid.

Abstract—Two new hexacyclic triterpenoids, isolated as their methyl esters from the roots of *Prunella vulgaris*, were established by spectroscopic evidence and chemical correlation to be (12*R*,13*S*)-2α,3α,24-trihydroxy-12,13-cyclo-taraxer-14-en-28-oic acid and (13*S*,14*R*)-2α,3α,24-trihydroxy-13,14-cyclo-olean-11-en-28-oic acid.

INTRODUCTION

We previously reported [1, 2] the structure determination of 13 pentacyclic triterpenes obtained as their methyl esters from *Prunella vulgaris* L. var. *lilacina* Nakai. Four of them were novel triterpenes, methyl 2α,3α-dihydroxyurs-12,20(30)-dien-28-oate, methyl 2α,3α,24-trihydroxyurs-12,20(30)-dien-28-oate(**1**), methyl 2α,3α,24-oleana-11,13(18)-dien-28-oate (**2**) and methyl 2α,3α,24-trihydroxyolean-12-en-28-oate (**3**), which were isolated together with some minor components by repeated preparative reversed-phase high performance liquid chromatography. In a continuation of that work, the two minor compounds have been identified as methyl (12*R*, 13*S*)-2α,3α,24-trihydroxy-12,13-cyclo-taraxer-14-en-28-oate (**4**) and methyl (13*S*,14*R*)-2α,3α,24-trihydroxy-13,14-cyclo-olean-11-en-28-oate (**5**), respectively.

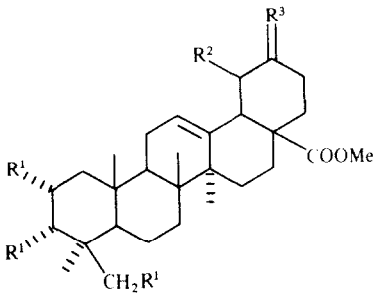
RESULTS AND DISCUSSION

The minor compounds **4** and **5** were effectively purified from the other four compounds **1**–**3** and methyl 2α,3α,24-trihydroxyurs-12-en-28-oate (**6**) by extensive HPLC of their trihydroxyl and triacetoxyl derivatives, respectively, using two mobile phases. The retention times of **4** and **5** were identical and were between those of the diene group (**1** and **2**) and the monoene group (**3** and **6**). The acetoxyl derivative **5a** behaved in a similar manner, however **4a** had a longer retention time than **3a** and consequently compound **4** was expected to have a unique structure.

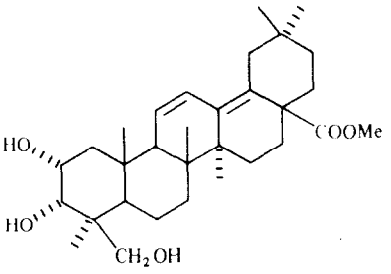
The molecular formulae $C_{31}H_{48}O_5$ of both compounds **4** and **5** were identical with those of **1** and **2**, and had two hydrogens less than those of **3** and **6**. A signal at *m/z* 239[M − retro-Diels–Alder fragment − H]$^+$ found in the mass spectra of **1**, **2**, **3** and **6** indicated that the A/B rings have the same structure. In fact, the ^{1}H NMR spectra of **4** and **5** (or **4a** and **5a**, see Table 1) showed the signals of four protons on the hydroxyl (or acetoxyl)-bearing carbons which were very similar to those of **3** (or **3a**) [2], revealing that they have the same substitution pattern. The differences were the presence of five tertiary methyl groups in the former, one less than in the latter and the appearance of double doublets at δ −0.09(in **4**) and a doublet at 0.32(in **5**), which absorbed at higher field than their methyl signals thus indicating a cyclopropane ring. The IR spectrum (*v* 3067 cm^{-1}) [3] of **4** supported the presence of a cyclopropane ring. The ^{13}C NMR spectra of **4** and **4a** suggested the location of the cyclopropane ring. Chemical shift differences between **3** and **4** were 90.4–120.7 ppm for C-12, C-13, C-14 and C-15, 4.6–14.5 ppm for C-7, C-16, C-18, C-19, C-26 and C-27, and less than 4.5 ppm for other carbons. These data, together with the mass spectral fragment ion described above, inferred that **4** differed from **3** only in the ring C and D region. Furthermore, the ^{1}H NMR (except for carbinyl region) and UV (λ_{max} 214 nm) spectra of **4** showed a marked resemblance to those of cyclosenegin[4]. Since it was known to undergo a vinylcyclopropane-homoallyl rearrangement, **4** was heated with 6 N hydrochloric acid in ethanol to afford the chloro derivative(**7**), whose ^{1}H NMR spectrum was again similar to that of senegenin[5, 6]. Thus the cyclopropane ring in **4** was likely to be fused to the C(12)–C(13) position of a nor-oleanane carbon skeleton.

In order to verify the above deduction, homo- and hetero-nuclear two dimension spectra were measured. These data resolved the cross-peaks corresponding to the coupling between 12-H and the geminal 27A-H and 27B-H, 15-H and 16-H_2, 18-H and 19-H_2, etc., together with the long-range coupling of 1β-H and 3β-H, 1α-H and 25-H_3, and 24-H_2 and 23-H_3. The unusual high field signal at δ −0.09 was thus due to 27A-H, which suffered for steric reasons the shielding effect of the C(14)–C(15) double bond. Though the 27B-H signal was overlapped with those of 25-H_3, 26-H_3, 29-H_3, 9-H and 19α-H, its coupling pattern was determined as δ0.86(*dd*, J_{12} = 9.0 Hz, J_{27A} = 4.5 Hz) by using *J*-resolved 2D NMR of **4**. Furthermore, the equatorial 19β-H(δ 0.64) and axial 9-

*Part 3 in the series 'Constituents of the Labiatae Plants'. For Part 2 see ref.[2].



	R^1	R^2	R^3
1	OH	Me	CH_2
3	OH	H	
3a	OAc	H	
6	OH	Me	
6a	OAc	Me	



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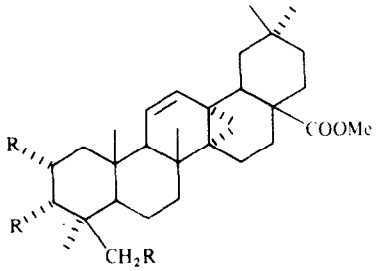
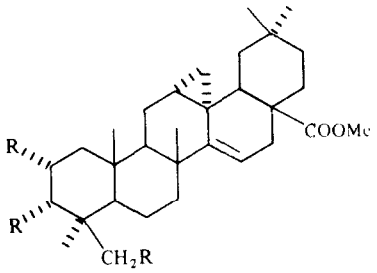
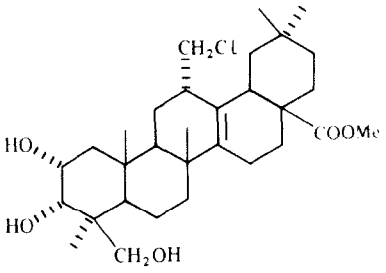


5 R = OH
5a R = OAc



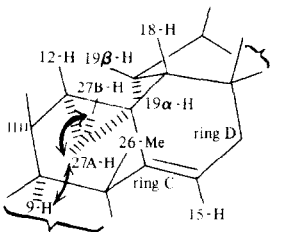
4 R = OH
4a R = OAc



7

H appeared at higher field than the axial 19α-H and 5-H, respectively, and than the same protons of **3**(Δ^5H: 0.8 ~ 0.9 ppm) probably due to their location (above and below the plane of the cyclopropane ring). This assumption was also supported by presence of 4 and 8% NOEs between 9-H and 27A-H, and 27A-H and 27B-H signals, respectively (indicated by arrows in Fig. 1). From these spectral data, the configuration of the cyclopropane ring was concluded to be *cis* to 9-H. Therefore, the compound **4** should be named methyl (12*R*,13*S*)-2α,3α,24-trihydroxy-12,13-cyclo-taraxer-14-en-28-oate. Phyllanthol, the first naturally occurring hexacyclic triterpene containing a cyclopropane ring reported by Barton *et al.* [7] possesses a modified ursane carbon skeleton.

The UV spectrum (λ_{max} 216 nm) of compound **5** was indicated the presence of a vinylcyclopropane. At the same time, two two-proton doublets (δ 0.35 and 1.08, $J = 5$ Hz) of a cyclopropyl methylene and the vicinal coupling of two vinylic protons (δ 5.14 and 5.84, $J = 11$ Hz) were observed in the ^{1}H NMR spectrum of **5a**. Moreover, chemical shift differences of the ^{13}C NMR spectra between **3** and **5** were 113.7–114.3 ppm for C-11 and C-13, 4.8–12.7 ppm for C-8, C-9, C-18, C-19 and C-27, and less than 4.1 ppm for other carbons, and between **4** and **5** were 94.4–127.1 ppm for C-11, C-12, C-14 and C-15, 5.4–12.6 ppm for C-9, C-13, C-16, C-17, C-19 and C-26, and less than 4.2 ppm for others. In particular the C-9



Partial structure of compound **4**

Fig. 1.

Table 1. ^{1}H NMR spectral data of compounds **4**, **4a**, **5**, **5a** and **7** ($CDCl_3$)

Assignments	**4**	**4a***	**5**	**5a***	**7**
H-2β	4.00*ddd*	5.19*ddd*	4.13*ddd*	5.21*ddd*	4.02*m*
	(12, 5, 3)	(12, 5, 3)	(12, 5, 3)	(12, 5, 3)	(21)
H-3β	3.86*d*	5.32*d*	3.86*d*	5.31*d*	3.85*d*
	(3)	(3)	(3)	(3)	(2)
H-11			5.82*dd*	5.84*dd*	
			(10, 3)	(11, 3)	
H-12			5.17*dd*	5.14*dd*	2.35*m*
			(10, 2.5)	(11, 2.5)	(20)
H-15	5.55*dd*	5.58*dd*			2.18*ddd*
	(8, 4)	(8, 4)			(22, 11, 3)
H-16α	2.36*ddd*	2.38*dd*			
	(14, 8, 1)	(14, 8)			
H-18	2.60*dd*	2.61*dd*	2.66*dd*	2.68*dd*	2.43*dd*
	(14, 4)	(14, 4)	(13, 4)	(13, 4)	(13, 4)
H-19β	0.64*dd*	0.63*dd*			
	(14, 4)	(14, 4)			
3H-23	1.13*s*	0.94*s*	1.13*s*	0.94*s*	1.13*s*
H_A and H_B	3.51*d*	4.04*d*	3.52*d*	4.07*d*	3.57*d*
	(11)	(11)	(11)	(11)	(11)
(or 3H)-24	3.70*d*	4.17*d*	3.66*d*	4.16*d*	3.67*d*
	(11)	(11)	(11)	(11)	(11)
3H-25	0.87*s*	1.01*s*	0.88*s*	0.99*s*	0.86*s*
3H-26	0.91*s*	0.88*s*	0.63*s*	0.66*s*	0.83*s*
H_A and H_B	-0.09*dd*	-0.09*t*	0.32*d*	0.35*d*	3.37*t*
	(6, 4.5)	(4.5)	(5)	(5)	(11)
-27	0.86*dd*		1.06	1.08*d*	3.75*dd*
	(9, 4.5)			(5)	(11, 3)
3H-29	0.89*s*	0.88*s*	0.91*s*	0.92*s*	0.91*s*
3H-30	0.90*s*	0.88*s*	0.94*s*	0.95*s*	1.01*s*
OMe	3.60*s*	3.60*s*	3.62*s*	3.65s	3.58s
OAc	—	1.95*s*	—	1.97*s*	—
		2.08*s*		2.09*s*	
		2.08*s*		2.12*s*	

*Measured at 300 MHz; the rest at 400 MHz.

—Indicates no signal. The figures in parentheses are coupling constants in Hz except for $W_{1/2}$ values in *m*.

signal was shifted downfield (Δ^δC: both 5.6 ppm) by comparison to the same signals of **3** and **4**, respectively, because it suffered the deshielding effect of the C(11)–C(12) double bond. Therefore, the cyclopropane ring was considered to be in the C(13)–C(14) position. These assignments were performed by comparison of the proton–proton and proton–carbon 2D NMR spectra data of **5** and **5a** with those of **3** and **4**, and their acetates **3a** and **4a**. It was very easy to identify the cross-peaks corresponding to the coupling between 27A-H and 27B-H, 18-H and 19-H_2, 9-H and 11-H, 11-H and 12-H, 1β-H and 3β-H (long-range coupling), *etc.* The coupling pattern of 18-H and 19-H_2 in **5** was similar to that of **3** and not so strange as that of **4**. Besides the above data, the configuration of the cyclopropane ring in **5** was suggested to be the same as **4** (*cis* to 9-H) by their biosynthetic relationship along with the oxidation and rearrangement of the C-27 methyl residue of an oleanene triterpene [6]. Therefore, compound **5** could be elucidated as an isomer of **4**, which had the cyclopropane ring located on the same position as that of phyllanthol [7]. Accordingly, it was named methyl (13*S*, 14*R*)-2α,3α,24-trihydroxy-13,14-cyclo-olean-11-en-28-oate.

EXPERIMENTAL

Mps: uncorr; TLC, IR, UV, MS. ^{1}H NMR, ^{13}C NMR, HPLC (except for mobile phase and flow rate), plant, extraction and isolation: see refs [1, 2].

Isolation of compounds **4** *and* **5** *by HPLC.* Each fraction contained minor compounds **4a** (29.2 min) and **5a** (26.0 min) which could be separated by HPLC[mobile phase MeCH–H_2O (87:13); flow rate (3.6 ml/min)] but the former fraction was a mixture of **3a**, **4a** and **6a** and the latter was a mixture of **3a** and **5a**. They were then, respectively, hydrolysed in 5% KOH–MeOH to yield the acetyl-free compounds. Compound **4** (22.8 min) was purified from **3** (24.5 min) and **6** (25.5 min) and compound **5** (22.8 min) was separated from **3** (24.5 min) by extensive HPLC [mobile phase MeOH–H_2O (4:1); flow rate 3.5 ml/min], respectively.

Methyl (12*R*,13*S*)-2α,3α,24-*trihydroxy*-12,13-*cyclo-taraxer*-14-*en*-28-*oate* (**4**). Recrystallization (MeOH) gave 35 mg, colourless

Table 2. ^{13}C NMR spectral data of compounds **3**, **4**, **4a**, **5**, **5a** and **7** ($CDCl_3$)

C	3*	4	4a*	5	5a*	7
1	41.4	41.6	38.6	41.9	38.7	41.5
2	66.2	66.3	67.8	66.2	67.5	66.5
3	73.3	73.2	72.3	73.5	72.4	73.5
4	41.4	43.9	42.0	43.9	42.0	43.9
5	48.6	48.7	50.2	48.3	49.8	48.6
6	18.2	18.2	18.0	18.4	18.0	18.4
7	32.7	38.7	38.6	36.8	36.5	38.4
8	39.3	37.1	37.0	34.1	34.0	38.9
9	47.4	47.4	47.7	53.0	52.9	51.3
10	38.0	38.0	38.1	37.5	37.5	38.0
11	23.4	19.0	19.1	137.7	137.9	20.8
12	121.9	14.9	14.7	119.9	119.3	44.9
13	141.7	23.0	23.0	30.0	29.9	143.5
14	41.6	156.0	156.1	28.9	28.9	130.3
15	27.5	117.9	118.1	23.5	23.3	20.0
16	22.9	31.8	31.9	21.4	21.3	21.1
17	46.6	52.8	52.8	45.0	44.8	46.7
18	41.1	34.7	34.6	34.7	34.5	39.1
19	45.7	35.5	35.4	40.9	40.7	42.0
20	30.6	28.7	28.7	32.9	32.9	30.8
21	33.7	33.6	33.6	34.1	34.0	33.4
22	32.2	30.4	30.4	32.9	32.8	30.6
23	22.0	22.1	22.2	22.1	22.0	22.1
24	65.5	65.4	66.0	65.3	65.8	65.5
25	16.6	17.6	17.5	18.5	18.3	17.5
26	16.6	28.9	28.9	16.3	16.2	19.1
27	25.9	11.4	11.4	15.6	15.6	47.7
28	178.2	178.1	177.9	178.1	177.9	178.0
29	33.0	32.1	32.1	32.7	32.6	32.9
30	23.5	22.6	22.6	24.3	24.1	24.9
$CO_2\underline{Me}$	51.4	51.5	51.5	51.7	51.6	51.5
<u>Me</u>CO			171.0		171.1	
			170.3		170.3	
			170.1		170.1	
<u>A</u>cCO			20.9		20.9	
			20.8		20.8	
			20.8		20.8	

*Measured at 75.2 MHz; the rest at 100 MHz.
†The data of **3** is cited from ref.[2].

needles, mp 276–278°, $[\alpha]_D^{24}$ +24.0° ($CHCl_3$; *c* 0.5); HRMS *m/z*: 500.348[M]$^+$, $C_{31}H_{48}O_5$ (required: 500.350); EIMS *m/z* (rel. int.): 500[M]$^+$ (67), 440[M − HCOOMe]$^+$ (25), 255(22), 241(27), 239(25), 213(19), 203(16), 200(100), 187(60), 133(19), 131(19); IR ν_{max} cm^{-1}: 3545(OH) 3067(cyclopropyl C-H), 1725(CO_2Me), 1635, 823 (C=CH): UV λ_{max} nm: 214 (log ε 3.67). *Triacetate* (**4a**), amorphous; HRMS *m/z* 626.382[M]$^{+\cdot}$ $C_{37}H_{54}O_8$ (required: 626.382); EIMS *m/z* (rel. int.): 626[M]$^+$ (100), 566(23), 387(11). 262(6), 200(73), 187(38), 133(5), ^1H NMR: see Table 1.; ^{13}C NMR: see Table 2.

Methyl (13*S*, 14*R*)-2α,3α,24-*trihydroxy*-13,14-*cyclo*-*olean*-11-*en*-28-*oate*(**5**). Crystallization of **5** from MeOH afforded 9 mg of colourless needles, mp > 310°, $[\alpha]_D^{24}$ +21.2° ($CHCl_3$; *c* 0.17); HRMS *m/z*: 500.349[M]$^+$, $C_{31}H_{48}O_5$ (required: 500.350); EIMS *m/z* (rel. int.): 500[M]$^+$(28), 440[M − HCOOMe]$^+$(6), 262(100), 239(10), 203(93), 189(13), 133(5); UV λ_{max} nm: 216(log ε 3.56). *Triacetate* (**5a**) was an amorphous powder. HRMS *m/z* 626.382[M]$^+$, $C_{37}H_{54}O_8$ (required: 626.382); EIMS *m/z* (rel. int.): 626[M]$^+$ (100), 566[M − HCOOMe]$^+$(34), 507(29), 431 (26), 371(18), 299(29), 262(33), 247(27), 239(23), 203(47), 189(35), 187(36), 133(28).

Treatment of **4** *with hydrochloric acid.* Compound **4** (15 mg) was refluxed with 6 N HCl in EtOH for 4 hr to give a residue, which was purified by HPLC [mobile phase MeOH–H_2O (92:8)] to yield **7**, *ca* 9 mg. stout needles (from MeOH), mp 152–153°, $[\alpha]_D^{25}$ −4.4° ($CHCl_3$; *c*0.3); FDMS *m/z*: 536[M]$^+$ EIMS *m/z* (rel. int.): 500[M − HCl]$^+$ (100), 482[M − HCl − H_2O]$^+$ (55), 440[M − HCl − HCOOMe]$^+$ (19), 255(6), 241(7), 239(5), 200(32), 187(16). Attempted conversion of **7** to **4** by the same procedure described in ref. [4] was unsuccessful, the starting material being recovered.

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