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TRITERPENOIDS FROM *PRUNELLA VULGARIS**

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Key Word Index—*Prunella vulgaris*; Labiatae; sterols; ursane triterpenoids; oleane triterpenoids; 2α,3α,24-trihydroxyolean-12-en-28-oic acid.

Abstract—Besides two sterols and three ursane and four oleane triterpenes already known, a new triterpene, 2α,3α,24-trihydroxyolean-12-en-28-oic acid has been isolated from the leaves and stems of *Prunella vulgaris*. ^{1}H and ^{13}C NMR assignments are given for ring A hydroxylated triterpenes.

INTRODUCTION

Prunella vulgaris L. var. *lilacina* Nakai is a perennial herb which is widely distributed throughout Japan, and has been used in the folk medicine as a diuretic. In Japan only its spike in the flowering period is used [1], but in China its stalk and leaves have been utilized [2] and in Europe the whole herb has been used [3]. The isolation of ursolic acid [4, 5] and oleanolic acid [5] has been reported. The present paper deals with the isolation and the identification of a novel 24-oxygenated oleane triterpene, and the assignment is described of the ^{13}C NMR spectral signals of the four ring-A substituted triterpenes. The ^{13}C NMR assignment was performed in comparison with the published spectral data of methyl maslinate, methyl 2α-hydroxyursolate and methyl 3-epimaslinate [6].

RESULTS AND DISCUSSION

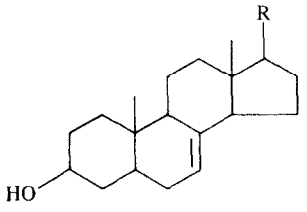
The methanol extract of the leaves and stems of *P. vulgaris* L. var. *lilacina* Nakai was diluted with water and then extracted with *n*-hexane, chloroform and *n*-butanol, successively. The *n*-hexane-soluble portion was fractionated by silica gel column to give a mixture of α-spinasterol (**1**) and stigmast-7-enol (**2**). From the chloroform-soluble portion was obtained a mixture of triterpenes, which was chromatographed on a silica gel column, and five fractions were separated. Each of the five fractions was treated with diazomethane, rechromatographed on a silica gel column yielding a portion which contained methyl ursolate (**3**) and methyl oleanolate (**4**), methyl 2α,3α-dihydroxyursan-12-en-28-oate (**5**) and methyl 3-epimaslinate (**6**), methyl 2α-hydroxyursolate (**7**) and methyl maslinate (**8**), methyl 2α,3α,23-trihydroxyolean-12-en-28-oate (**9**), and a new triterpene (**10**). Compounds **3**, **5**, **9** and **10** were purified by recrystallization.

Relative amounts of **1** and **2**, **3** and **4**, **5** and **6**, and **7** and **8** in each fraction were confirmed by GC and/or GC/MS. The structures of these compounds were determined by means of GC, GC/MS and NMR spectroscopy. Their data were compared with those of authentic or synthetic samples, when available (see Experimental).

High-resolution mass spectrometry revealed that the molecular formula of **10** was $C_{31}H_{50}O_5$, which was the same as that of **9** and had one more oxygen than compounds **5**, **7** and **8**. On acetylation, compounds **5**, **9** and **10** gave diacetate **5a** and triacetates **9a** and **10a**, whose IR spectra showed no hydroxyl absorption. Therefore, their oxygens could be attributed to one carboxyl and two hydroxyls in **5**, and one carboxyl and three hydroxyls in **9** and **10**. Abundant ions in their mass spectra were at *m/z* 262 and 203 usually resulting from the typical retro-Diels–Alder cleavage of ring C of ursan-12-enes and olean-12-enes with a C-17 methoxycarbonyl and with no hydroxyl groups on rings D/E [7]. Further, the appearance of a signal at *m/z* 223 (ion **a**) in **5** [8] and *m/z* 239 (ion **b**) in **10** indicated that the additional oxygen atoms were present on rings A/B.

In comparison with the ^{1}H NMR spectra of **5**, **9**, **10** and methyl asiatate (**11**) which was isolated from *Syzygium* sp. [9], each compound showed a one-proton triplet on a trisubstituted double bond around δ5.2 and a singlet due to a carboxymethyl group at δ3.5–3.6. In addition, the one-proton signal due to an allylic 18β-hydrogen was observed as a doublet near δ2.2 in **5** and **11** and as a doublet of doublets at δ2.8 in **9** and **10**, which exactly indicated the difference between a methyl urs-12-en-28-oate and the oleanene analogue [10]. These results were in agreement with the data of the ^{13}C NMR spectra (Table 1), which clearly exhibited the difference in the chemical shifts of C-12, C-13, C-17, C-18, C-19, C-20, C-22, C-27, C-29 and C-30 between the ursane group (**5**, **7** and **11**) and the oleane group (**8**, **9** and **10**). The ^{1}H NMR data of their acetyl derivatives showed the presence of two vicinal secondary acetoxyl groups in all derivatives and one primary acetoxyl group in **9a**, **10a** and **11a**. Periodic acid titration of compound **10** also showed that the two hydroxyl groups were vicinal to each other. By analogy with many other cases, a 2,3-diol is very likely, so the different splitting patterns of two protons between compounds **5a**, **9a** and **10a** (δ5.1–5.2, *m*, $W_{1/2}$ = 22 Hz and δ4.7–5.1, *d*, *J* = 3 Hz) and a mixture of **7a** and **8a** and **11a**

*Part 1 in the series "Constituents of the Labiate Plants".



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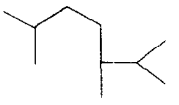

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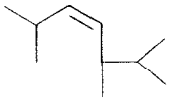
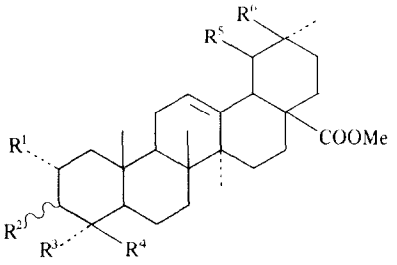
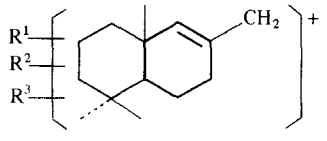




	R^1	R^2	R^3	R^4	R^5	R^6
3	H	βOH	Me	Me	Me	H
4	H	βOH	Me	Me	H	Me
5	OH	αOH	Me	Me	Me	H
5a	OAc	αOAc	Me	Me	Me	H
6	OH	αOH	Me	Me	H	Me
7	OH	βOH	Me	Me	Me	H
7a	OAc	βOAc	Me	Me	Me	H
8	OH	βOH	Me	Me	H	Me
8a	OAc	βOAc	Me	Me	H	Me
9	OH	αOH	CH_2OH	Me	H	Me
9a	OAc	αOAc	CH_2OAc	Me	H	Me
10	OH	αOH	Me	CH_2OH	H	Me
10a	OAc	αOAc	Me	CH_2OAc	H	Me
11	OH	βOH	CH_2OH	Me	Me	H
11a	OAc	βOAc	CH_2OAc	Me	Me	H



a *m/z* 223 $R^1 = R^2 = OH$, $R^3 = H$
b *m/z* 239 $R^1 = R^2 = R^3 = OH$

(δ5.1–5.2, *m*, $W_{1/2}$ = 22 Hz and δ4.7–5.0, *d*, J = 11 Hz) indicated as expected that the vicinal coupling of the former group was due to the protons (ax-eq) on C-2β and C-3β [10]. Therefore, the C-2 and C-3 hydroxyl groups of the former were α-equatorial and α-axial, respectively. Moreover, the different appearances of the two two-proton doublets between **9a** (δ3.71 and 4.04, J = 12 Hz) and **11a** (δ3.54 and 3.86, J = 12 Hz) on the one hand and **10a** (δ3.96 and 4.16, J = 12 Hz) showed that each C-4 acetoxymethylene in the former pair was at C-23 (eq) since an equatorial one resonates at higher field than δ3.9 [11], whereas that of **10a** was at C-24 (ax). The difference between the 2α,3β-dihydroxyl group (**7**, **8** and **11**) and the 2α,3α-dihydroxyl group (**5**, **9** and **10**) appeared in the chemical shifts of C-1, C-2, C-3 and C-5 in the ^{13}C NMR spectra. In particular, C-5 and C-1 in the latter were largely shifted to higher field by the axial 3α-hydroxyl (γ-effect). Further, the large upfield shift of C-5 shown by **9** and **11** was also given by the C-23 primary alcoholic function, which at the same time shielded C-24, whereas the hydroxyl at C-24 similarly affected C-23 but deshielded C-5 to only a small extent.

Table 1. ^{13}C NMR spectral data of compounds **5**, **9**, **10** and **11** (25.2 MHz, $CDCl_3$)

Carbon	5	6*	7*	8*	9	10	11
1	42.2	41.7	46.8	46.4	41.7	42.0	46.7
2	66.7	66.5	68.9	68.8	66.7	66.6	69.0
3	79.2	78.9	83.8	83.8	78.8	73.8	79.9
4	39.3†	38.5†	39.1	39.1	41.1	44.2	42.9
5	48.3	48.1	55.4	55.3	42.6	49.0	48.8
6	18.2	18.1	18.4	18.3	18.0	18.6	18.4
7	33.0	32.5	32.9	32.6	32.6	32.6	32.8
8	40.0†	39.7†	39.6	39.1	39.7	39.8	39.8
9	47.6	47.4	47.5	47.5	47.7	47.8	47.7
10	38.4	38.3†	38.3	38.3	38.2	38.4	38.3
11	23.4	23.4	23.4	23.5	23.2	23.3	23.5
12	125.8	122.1	125.3	122.0	122.4	122.5	125.7
13	138.7	143.8	138.1	143.6	144.4	144.3	138.7
14	42.2	41.9	42.1	41.7	42.0	42.0	42.3
15	28.2	27.7	28.0	27.6	27.9	27.9	28.2
16	24.4	23.2	24.3	23.1	23.6	22.2	24.4
17	48.3	46.8	48.1	46.6	47.0	47.0	48.3
18	53.2	41.3	52.8	41.3	41.6	41.6	53.1
19	39.1	46.0	39.1	45.8	46.2	46.2	39.3
20	38.5	30.7	38.9	30.7	30.8	30.9	39.1
21	30.8	34.0	30.7	33.8	34.1	34.1	30.9
22	36.8	32.5	36.7	32.3	32.4	32.6	36.8
23	28.6	28.5	28.7	28.6	71.5	23.7	69.3
24	22.0	21.9	17.0	16.8	17.5	66.0	13.1
25	16.5	16.4	17.0	16.8	16.8	16.8	17.1
26	17.1	17.0	17.0	16.8	17.1	16.9	17.1
27	23.9	26.2	23.7	26.0	26.2	26.2	23.9
28	178.4	178.1	177.9	178.0	178.7	178.7	178.4
29	17.1	33.2	17.0	33.1	33.2	33.2	17.3
30	21.2	23.6	21.2	23.5	23.8	23.7	21.2
$CO_2\underline{Me}$	51.4	51.5	51.5	51.5	51.5	51.6	51.5

*The data of **6**, **7** and **8** are cited from ref. [6].
†Assignments may be reversed, pairwise.

From these data, the compounds **5**, **9** and **10** can be assigned as methyl 2α,3α-dihydroxyursan-12-en-28-oate, methyl 2α,3α,23-trihydroxyolean-12-en-28-oate and methyl 2α,3α,24-trihydroxyolean-12-en-28-oate, respectively. This is the first report of 2α,3α-dihydroxyursan-12-en-28-oic acid as a natural constituent of the Labiatae. 2α,3α,23-Trihydroxyolean-12-en-28-oic acid has been isolated only once before [12] and the ursane equivalent of **10**, 2α,3α,24-trihydroxyurs-12-en-28-oic acid has been found in nature [13].

EXPERIMENTAL

All mps are uncorr. IR spectra were recorded on KBr discs. ^1H NMR spectra (in $CDCl_3$) were run at 90 MHz and ^{13}C NMR at 25.2 MHz with TMS as internal standard. MS (20 eV) were taken with a direct inlet. GC was run with operating conditions GC (1) for phytosterols: U-shaped glass column (2.0 m × 3 mm) packed with 1.5% OV-1: column temp. 260°: N_2 at 40 ml/min; or GC (2) for triterpene methyl esters: column (0.5 m × 3 mm) packed with 2% OV-17: column temp. and N_2 flow rate, unless otherwise indicated, 275° and at 38 ml/min. GC/MS was done in the same way as described above.

Plant material. The plant *P. vulgaris* var. *lilacina* was transplanted from Omachi, Nagano Prefecture to Medicinal Plant Garden (School of Pharmaceutical Sciences, Kitasato University) in 1978 and collected from the Garden in June 1980.

Extraction and isolation. The dried cut leaves and stems (0.7 kg) were extracted with MeOH to give an extract (96.4 g), which was suspended in H_2O and extracted with *n*-hexane, $CHCl_3$ and *n*-BuOH, successively. The *n*-hexane (8.7 g) extract was fractionated on a silica gel column. From the C_6H_6 eluate was obtained a steroidal powder (120 mg). The $CHCl_3$ extract (26.0 g) was examined by TLC on silica gel ($CHCl_3$–Me_2CO, 1:1), detection with 10% H_2SO_4; R_f 0.68 (red-violet), 0.63 (violet-brown), 0.57 (brown), 0.53 (blue), 0.48 (brown), 0.22 (light brown), 0.12 (violet). This extract was chromatographed on silica gel to give the two major constituents in the $CHCl_3$–Me_2CO (9:1) eluate and three other fractions from the 17:3, 4:1 and 1:1 eluates. Each of the five fractions was esterified with CH_2N_2 to give a solid product which was purified by repeated silica gel CC to afford the five mixtures, which contained **3** and **4** (747 mg), **5** and **6** (183 mg), **7** and **8** (140 mg), **9** (80 mg), and **10** (77 mg), respectively.

α-Spinasterol (**1**) *and stigmast-7-enol* (**2**). The powder was crystallized from MeOH to afford 51 mg of colourless needles, mp 155–157°, $[\alpha]_D^{23}$ + 3.0° ($CHCl_3$; *c* 1); IR ν_{max} cm^{-1}: 3440 (OH), 1664 (C=C), 1460 (CH), 833 (C=CH); ^1H NMR: δ0.56 (*s*,

18-H_3), 3.59 (*m*, $W_{1/2}$ = 22 Hz, H-3), 5.12 (*m*, vinyl protons). These data showed that the product was a mixture of unknown (R_t 13.2 min and rel. amount 1.7% by GC (1) and $[M]^+$ *m/z* 400 by GC/MS), **1** (14.3; 56.3; 412) and **2** (16.1; 41.9; 414). Data of **1** and **2** were identical with those of corresponding authentic samples isolated from *Bupleurum falcatum* L. [14, 15] (co-TLC and co-GC).

Methyl ursolate (**3**) *and methyl oleanolate* (**4**). An amorphous solid was found to be identical with the mixture of authentic samples by co-TLC and co-GC (2): column temp. 260°, N_2 at 33 ml/min, **3** (R_t 7.3 min and rel. amount 93.6%), **4** (6.3; 6.4) and the fragmentation patterns by GC/MS. The residue was recrystallized from MeOH to give 211 mg of colourless needles, mp 170–172°, which was identified as methyl ursolate.

Methyl 2α,3α-dihydroxyurs-12-en-28-oate (**5**) *and methyl 3-epimaslinate* (**6**). From the results of GC (2) and GC/MS, compound **5** (R_t 6.1 min, rel. amount 96.6% and $[M]^+$ *m/z* 486), **6** (5.3; 1.3; 486) and unknown (7.3; 2.1). R_f (TLC), R_t (GC) and fragmentation pattern (GC/MS) of **6** were identical with that of an authentic sample isolated from tissue cultures of *Isodon japonicus* Hara [6]. The residue was recrystallized from MeOH to give 34 mg of colourless needles, mp 186–187°, $[\alpha]_D^{26}$ + 58.4° ($CHCl_3$; *c* 0.5). HRMS: *m/z* 486.372 $[M]^+$, calc. for $C_{31}H_{50}O_4$, 486.371. EIMS: *m/z* (rel. int.); 486 $[M]^+$ (6), 262 (100), 249 (15), 223 (13), 203 (61), 189 (13), 133 (19). IR ν_{max} cm^{-1}: 3525 (OH), 1724 (CO_2Me), 1030 (OH), 827 (C=C). ^1H NMR: δ0.73 (*s*, 26-H_3), 0.84 (*s*, 24-H_3), 0.85–0.93 (2*d*, 29-H_3 and 30-H_3), 0.94 (*s*, 25-H_3), 1.01 (*s*, 23-H_3), 1.07 (*s*, 27-H_3), 2.22 (*d*, *J* = 11 Hz, H-18), 3.40 (*d*, *J* = 3 Hz, H-3), 3.56 (*s*, CO_2Me), 3.97 (*m*, $W_{1/2}$ = 22 Hz, H-2), 5.22 (*t*, *J* = 4 Hz, H-12). These data were identical with those of the synthetic main product prepared by OsO_4-oxidation of methyl urs-2,12-dien-28-oate, which was derived from dehydration of **3** [10, 16].

Diacetate of compound **5** (**5a**). Amorphous powder. High-resolution MS: *m/z* 570.392 $[M]^+$, calc. for $C_{35}H_{54}O_6$, 570.392. IR ν_{max} cm^{-1}: 1748 (OAc), 1724 (CO_2Me), 1600 (C=C), 827 (C=C). ^1H NMR: δ0.74 (*s*, 26-H_3), 0.85–0.95 (2*d*, 29-H_3 and 30-H_3), 0.87 (*s*, 23-H_3), 0.97 (*s*, 24-H_3), 1.03 (*s*, 25-H_3), 1.11 (*s*, 27-H_3), 1.92, 2.08 (2*s*, Ac × 2), 3.56 (*s*, CO_2Me), 4.92 (*d*, *J* = 3 Hz, H-3), 5.22 (*m*, $W_{1/2}$ = 22 Hz, H-2), 5.22 (*m*, H-12).

Methyl 2α-hydroxyursolate (**7**) *and methyl maslinate* (**8**). From the results of GC (2) and GC/MS, **7** (R_t 6.2 min; rel. amount 62.5%; $[M]^+$ *m/z* 486.370 calc. for $C_{31}H_{50}O_4$, 486.371) and **8** (5.4; 37.5%; 486.371). R_f (TLC), R_t (GC) and fragmentation patterns (GC/MS) of **7** and **8** were identical with those of authentic samples and the ^1H NMR spectrum was also assigned as that of their mixture: δ2.21 (*d*, *J* = 11 Hz, H-18) and 2.84 (*m*, H-18), 2.96 (*d*, *J* = 12 Hz, H-3), 3.56 (*s*) and 3.58 (*s*, CO_2Me) 3.66 (*m*, $W_{1/2}$ = 22 Hz, H-2), 5.21 (*t*) and 5.24 (*t*, H-12).

^1H *NMR of diacetate of a mixture of compounds* **7** *and* **8**(**7a** *and* **8a**), *methyl asiatate* (**11**) *and triacetate of* **11** (**11a**). ^1H NMR of a mixture of **7a** and **8a**: δ0.71–1.11 (methyl protons), 1.94, 2.02 (2*s*, Ac × 2), 2.83 (*dd*, *J* = 15, 5 Hz, H-18), 3.56, 3.58 (2*s*, CO_2Me × 2), 4.69 (*d*, *J* = 12 Hz, H-3), 5.19 (*m*, $W_{1/2}$ = 22 Hz, H-2), 5.19, 5.22 (2*t*, H-12). ^1H NMR of **11**: δ0.72 (*s*, 26-H_3), 0.78–1.01 (24, 25, 29 and 30-H_3), 1.07 (*s*, 27-H_3), 2.22 (*d*, *J* = 12 Hz, H-18), 3.56 (*s*, CO_2Me), 5.22 (*m*, H-12). ^1H NMR of **11a**: δ0.75 (*s*, 26-H_3), 0.86–1.09 (24, 25, 27, 29 and 30-H_3), 1.96, 2.00, 2.06 (3*s*, Ac × 3), 2.22 (*d*, *J* = 11 Hz, H-18), 3.54, 3.86 (2*d*, *J* = 12 Hz, H_A-23, H_B-23), 3.57 (*s*, CO_2Me), 5.02 (*d*, *J* = 10 Hz, H-3), 5.16 (*m*, $W_{1/2}$ = 22 Hz, H-2), 5.22 (*t*, *J* = 4, H-12).

Methyl 2α,3α,23-trihydroxyolean-12-en-28-oate (**9**). Crystallization from MeOH afforded 25 mg of colourless needles, mp 236–237°, $[\alpha]_D^{26}$ + 56.0° ($CHCl_3$; *c* 0.5). High-resolution MS: *m/z* 502.365 $[M]^+$, calc. for $C_{31}H_{50}O_5$, 502.366. EIMS *m/z* (rel. int.): 502 $[M]^+$ (5), 484 $[M - H_2O]^+$ (1), 442 $[M - HCO_2Me]^+$ (5), 262 (100), 249 (18), 203 (88), 189 (11), 133 (5). IR ν_{max} cm^{-1}: 1450 (OH), 1715 (CO_2Me), 1660 (C=C) 825 (C=C). ^1H NMR: δ0.72 (*br s*, 24, 26-H_3), 0.89 (*s*, 29-H_3), 0.92 (*s*, 30-H_3), 0.97 (*s*, 25-H_3), 1.14 (*s*, 27-H_3), 2.85 (*dd*, *J* = 15, 5 Hz, H-18), 3.3–3.8 (H-3, H_A-23 and H_B-23 unresolved signals), 3.58 (*s*, CO_2Me), 3.91 (*m*, $W_{1/2}$ = 22 Hz, H-2), 5.24 (*t*, *J* = 4 Hz, H-12). Identical with authentic sample isolated from *Pseudopanax arboreum* [12] (co-TLC, co-GC (2) and mmp).

Triacetate of compound **9** (**9a**). Amorphous. HRMS: *m/z* 628.400 $[M]^+$, calc. for $C_{37}H_{56}O_8$, 628.397. IR ν_{max} cm^{-1}: 1751 (OAc), 1715 (CO_2Me), 1660, 825. ^1H NMR: δ0.74 (*s*, 26-H_3), 0.90 (*s*, 29-H_3), 0.92 (*s*, 30-H_3), 1.07 (*s*, 24-H_3), 1.11 (*s*, 25-H_3), 1.17 (*s*, 27-H_3), 1.94, 1.98, 2.02 (3*s*, Ac × 3), 2.85 (*dd*, *J* = 15, 5 Hz, H-18), 3.59 (*s*, CO_2Me), 3.71, 4.04 (2*d*, *J* = 12 Hz, H_A-23 and H_B-23), 5.12 (*d*, *J* = 3 Hz, H-3), 5.18 (*m*, $W_{1/2}$ = 22 Hz, H-2), 5.25 (*t*, *J* = 4 Hz, H-12).

Methyl 2α,3α,24-trihydroxyolean-12-en-28-oate (**10**). Recrystallization from MeOH afforded 35 mg of colourless needles, mp 280–282°, $[\alpha]_D^{27}$ + 59.6° ($CHCl_3$; *c* 1). HRMS *m/z* 502.363 $[M]^+$, $C_{31}H_{50}O_5$ (required 502.366). EIMS *m/z* (rel. int.): 502 $[M]^+$ (3), 425 $[M - CO_2Me - H_2O]^+$ (3), 262 (83), 249 (8), 239 (9), 203 (100), 189 (19), 133 (8). IR ν_{max} cm^{-1}: 3511 (OH), 1726 (CO_2Me), 1660, 821. ^1H NMR: δ0.68 (*s*, 26-H_3), 0.91 (*br s*, 25, 29, 30-H_3), 1.12 (*br s*, 23, 27-H_3), 2.83 (*dd*, *J* = 15, 5 Hz, H-18), 3.41, 3.63 (2*d*, *J* = 12 Hz, H_A-24 and H_B-24, partly covered by signal due to methyl ester proton), 3.57 (*s*, CO_2Me), 3.81 (*d*, *J* = 3 Hz, H-3) 3.91 (*m*, $W_{1/2}$ = 22 Hz, H-2), 5.24 (*t*, *J* = 4 Hz, H-12).

Triacetate of compound **10** (**10a**). Amorphous. HRMS: *m/z* 628.398 $[M]^+$, $C_{37}H_{56}O_8$ (required 628.397). IR ν_{max} cm^{-1}: 1750 (OAc), 1726 (CO_2Me), 1660, 821. ^1H NMR: δ0.70 (*s*, 26-H_3), 0.88 (*br s*, 29, 30-H_3), 0.92 (*s*, 23-H_3), 1.01 (*s*, 25-H_3), 1.16 (*s*, 27-H_3), 1.92, 2.09, 2.12 (3*s*, Ac × 3), 2.82 (*dd*, *J* = 15, 5 Hz, H-18), 3.58 (*s*, CO_2Me), 3.97, 4.17 (2*d*, *J* = 12 Hz, H_A-24 and H_B-24), 5.13 (*m*, $W_{1/2}$ = 22 Hz, H-2), 5.22–5.23 (H-3, H-12 unresolved signals due to covering by each proton).

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