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Studies on the Constituents of *Beesia calthaefolia* and *Souliea vaginata*. IV.[1)] Beesioside I, a Cyclolanostanol Xyloside from the Rhizomes of *Beesia calthaefolia*

Nobuko SAKURAI,[a] Masahiro NAGAI,*[,a] Teruo GOTO,[a] Takao INOUE[a] and Pei-Gen XIAO[b]

Faculty of Pharmaceutical Sciences, Hoshi University,[a] Shinagawa-ku, Tokyo 142, Japan and Institute of Medicinal Plant Development,[b] Chinese Academy of Medical Sciences, Dong Bei Wang, Beijing, China. Received July 9, 1992

A new triterpenol xyloside, beesioside I was isolated from the rhizomes of *Beesia calthaefolia*, and the structure was elucidated as (20*S*,24*S*)-15*β*,16*β*-diacetoxy-18,24;20,24-diepoxy-9,19-cyclolanostane-3*β*,25-diol 3-*O*-*β*-D-xylopyranoside on the basis of chemical and physicochemical evidence, including nuclear Overhauser effect spectroscopy experiment.

Keywords *Beesia calthaefolia*; beesioside I; Ranunculaceae; 9,19-cyclolanostanol xyloside; nuclear Overhauser effect spectroscopy

Beesia calthaefolia MAXIM. (Ranunculaceae) has been used in China as a herbal drug for its anti-inflammatory and analgesic effects. In previous papers,[1,2)] we reported the structure elucidation of new triterpenol xylosides, beesioside II—IV, from the rhizomes of *B. calthaefolia* or *Souliea vaginata*. The present paper elucidates the structure of beesioside I on the basis of chemical and physicochemical evidence.

The extraction and separation of beesioside I (**1**) were described in the literature.[2a)] Beesioside I (**1**), mp 264—266 °C, $[\alpha]_D$ −10.7°, $C_{39}H_{60}O_{12} \cdot H_2O$, showed strong absorption bands of hydroxyl groups (3600—3200 cm^{-1}) and acetoxyl groups (1738, 1238 cm^{-1}) in its infrared (IR) spectrum. The proton and carbon 13-nuclear magnetic resonance (^1H- and ^{13}C-NMR) spectra indicated that **1** is a glycoside of 9,19-cyclolanostanol, having two acetoxyl groups (δ 2.11 (6H, s), δ 170.8, 170.5) and a ketal carbon (δ 114.1) (Table I).

Enzymatic hydrolysis of **1** with molsin yielded an aglycone (**2**), mp 188—190 °C, $[\alpha]_D$ −0.1° and xylose. The electron impact high resolution mass spectrum (EI-HRMS) of **2** gave a parent ion at *m/z* 588.3689 corresponding to a molecular formula $C_{34}H_{52}O_8$ requiring nine sites of unsaturation. The ^{13}C-NMR spectrum of **2** showed 34 distinct resonances attributable to the eight methyls, ten methylenes, six methines and ten nonprotonated carbon atoms [from insensitive nuclei enhanced by polarization transfer (INEPT) experiments], which accounted for 50 out of the 52 protons in the molecule. The remaining two protons should be part of the hydroxyl functions. The ^1H-NMR spectrum of **2** showed that **2** possesses a cyclopropane ring, six methyl groups, two acetoxyl groups, an ABX type of three methines (H-15, 16 and 17), an AB type methylene group (H_2-18) bearing an ethereal oxygen atom and a hydroxyl proton at δ 2.22 (which disappeared on addition of D_2O). The ^1H–^1H shift correlation spectrum (COSY) of **2** showed cross peaks between a signal at δ 5.92 (1H, dd, J=9.0, 11.5 Hz, H-16) and two methine signals at δ 5.68 (1H, d, J=9.0 Hz, H-15) and 2.71 (1H, d, J=11.5 Hz, H-17) (Table II).

Assignment of the three ABX methines was also confirmed by decoupling experiments. Irradiation of H-16 changed the two doublets due to H-15 and H-17 into two singlets. On irradiation of the H-15 or H-17, the H-16 signal changed into a doublet. From the ^1H–^{13}C COSY of **2**, ^{13}C-signals at δ 82.0, 75.1 and 56.2 were correlated with the ^1H-signals due to H-15, 16 and 17, respectively. Thus, the two acetoxyl groups in **2** are located at C-15 and C-16. These findings indicate **2** has a partial structure A (Chart 1).

TABLE I. ^{13}C-NMR Data of Beesioside I (**1**), **2**, **4** and **5** (in Pyridine-d_5)

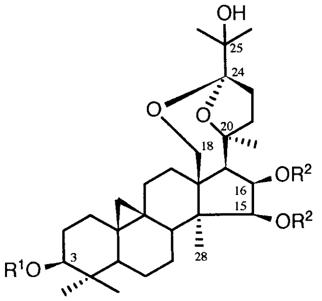
C	**1**	**2**	**4**	**5**
1	32.35	32.51	32.64	32.58
2	30.76	31.15	31.23	30.65
3	88.18	77.78	77.91	77.79
4	41.16	41.03	41.04	40.98
5	47.03	47.03	47.38	46.91
6	20.37	20.72	21.08	20.19
7	26.13	26.32	26.83	26.13
8	47.03	47.16	48.79	48.67
9	18.96	19.08	19.67	19.84
10	27.48	27.90	27.83	26.77
11	25.89	26.12	26.65	26.13
12	27.83	27.98	28.47	27.24
13	45.62	45.80	46.09	42.04
14	51.37	51.50	50.78	58.71
15	81.90	82.03	84.13	79.23
16	75.03	75.14	76.68	77.79
17	56.07	56.22	58.18	52.31
18	66.23	66.38	66.99	73.92
19	31.41	31.62	32.06	31.12
20	86.66	86.79	88.03	86.19
21	32.35	32.42	33.23	31.70
22	38.10	38.19	38.46	38.51
23	29.82	30.83	30.88	29.30
24	114.07	114.22	113.66	215.82
25	72.68	72.72	72.92	76.73
26	25.60	25.65	25.66	23.66
27	25.60	25.58	25.66	26.13
28	25.60	26.12	26.13	27.24
29	15.32	15.41	14.79	14.85
30	15.20	14.71	14.79	14.68
Ac	21.19	21.18		20.96
	21.19	21.21		20.96
	170.50	170.51		170.50
	170.79	170.82		170.84
1′	107.26			
2′	75.27			
3′	78.32			
4′	70.98			
5′	66.87			

In the ^{13}C-NMR spectrum of **1**, the signals at δ 107.3, 75.3, 78.3, 71.0 and 66.9 were assignable to the C-1—C-5 of β-D-xylopyranose. The glycosylation shift was observed for the C-3 signal (+10.4 ppm) of the aglycone (**2**), indicating the site of glycosylation. Application of Klyne's rule[3] to **1** and **2** also supported the β-D-xylopyranoside structure [molecular rotation difference at 589 nm between **1** and **2**, −79.6°; methyl β-D-xylopyranoside, $[M]_D$ −108°; methyl α-D-xylopyranoside, $[M]_D$ +253°[4]].

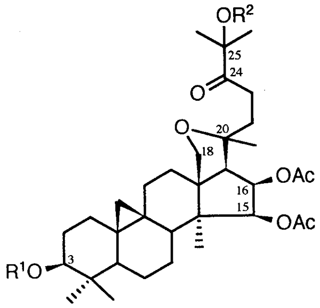
Acetylation of **2** with acetic anhydride afforded a triacetate (**3**), mp 214—215 °C, $C_{36}H_{54}O_9$. In the ^{1}H-NMR spectra, the signals due to H-3 showed a downfield shift

Table II. ^{1}H-NMR Data of Aglycone (**2**) (in Pyridine-d_5)

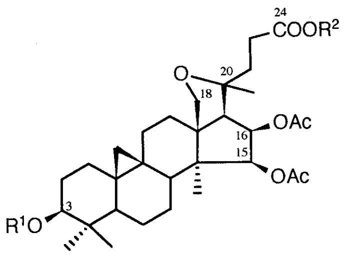
H		H	
1	*ca.* 1.27 m	17	2.71 d (11.5)
	1.55 m	18	4.50 d (12.9)
2	*ca.* 1.85 m		4.62 d (12.9)
	2.00 m	19	0.22 d (3.9)
3	3.54 dd (4.4, 11.7)		0.55 d (3.9)
5	1.28 m	21	1.28 s
6	0.67 q (12.2)	22	*ca.* 1.96 m
	1.48 m		2.98 m
7	1.27 m	23	*ca.* 2.07 m
	2.05 m		2.78 m
8	1.55 m	26	1.65 s
11	*ca.* 1.27 m	27	1.54 s
	1.55 m	28	1.21 s
12	*ca.* 1.55 m	29	1.21 s
	2.94 m	30	1.04 s
15	5.68 d (9.0)	Ac	2.11 s
16	5.92 dd (9.0, 11.5)		2.12 s



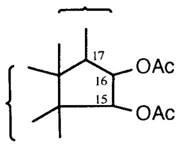
1 : R^1=xyl(p), R^2=Ac
2 : R^1=H, R^2=Ac
3 : R^1,R^2=Ac
4 : R^1,R^2=H



5 : R^1,R^2=H
6 : R^1=Ac, R^2=H
7 : R^1,R^2=Ac



8 : R^1,R^2=H
9 : R^1=H, R^2=Me
10 : R^1=Ac, R^2=Me



A

Chart 1

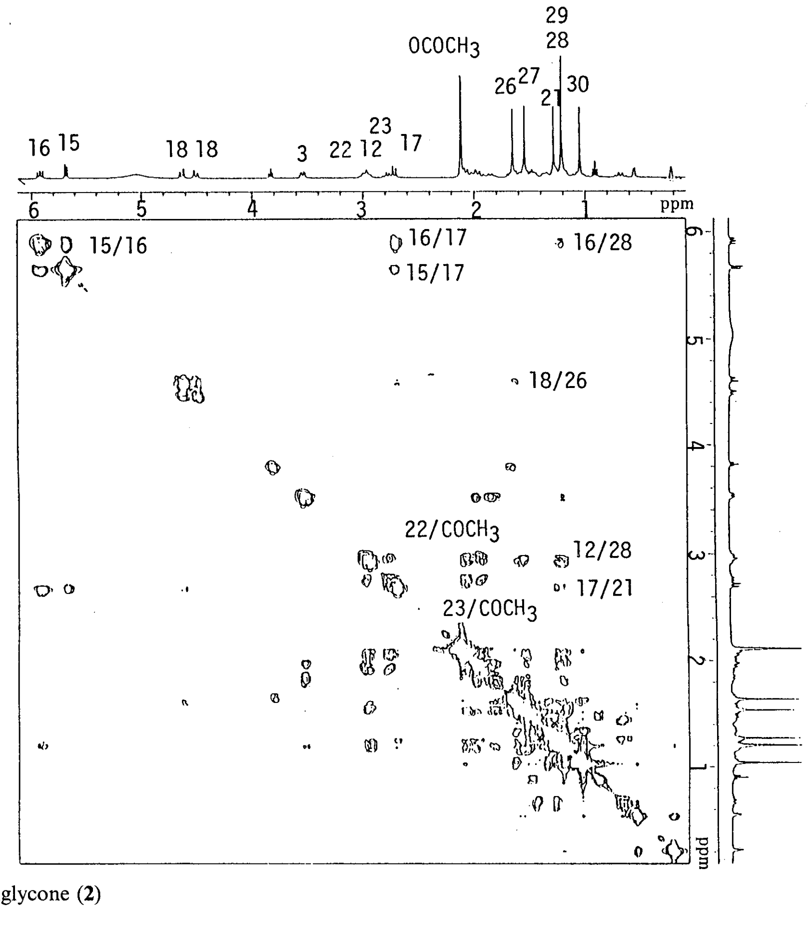


Fig. 1. NOE Spectrum of Aglycone (**2**)

from δ 3.54 in **2** to δ 4.53 in **3**.

Alkaline treatment of **2** with 2.5% KOH in EtOH gave compound **4**, mp 159—160 °C, $C_{30}H_{48}O_6$. In the ^{1}H-NMR spectra, the signals due to H-15 and H-16 showed upfield shifts from δ 5.68 and 5.92 in **2** to δ 3.86 and 4.10 in **4**, respectively, indicating two acetoxyl groups linked to C-15 and C-16.

On treatment with *p*-toluenesulfonic acid, **2** gave compound **5**, mp 134—136 °C, $C_{34}H_{52}O_8$. The IR spectrum of **5** unexpectedly showed the absorption due to a carbonyl group at 1703 cm^{-1}. In the ^{13}C-NMR spectrum of **5**, the signal due to a carbonyl group was at δ 215.8, indicating that **5** has a ketonic function. EI-MS of **5** gave a fragment ion peak at *m/z* 473 $[M-CH_2CH_2COC(CH_3)_2OH]^+$. When aglycone (**2**) was treated with the acid, the cleavage of the ketal at C-24 in **2** and subsequent five membered ether ring formation between the two carbinol groups at C-18 and C-20, explained satisfactorily the formation of the ketonic compound **5**. The stereochemistry at C-20 of **5** remains undetermined, because of the probable reaction mechanism to the ether formation between a primary alcohol at C-18 and a tertiary alcohol at C-20 under the acidic condition.

Alkaline treatment followed by acetylation of **5** gave a triacetate (**6**), mp 161—162 °C, and a tetraacetate (**7**) (minor product). Acid hydrolysis followed by acetylation of beesioside I (**1**) also gave the same compounds **6** and **7**.

On treatment with sodium metaperiodate and cyclohexylamine in methanol, **5** afforded an acid (**8**), mp 240—242 °C, $C_{31}H_{46}O_8$. In the ^{1}H-NMR spectrum of **8**, signals due to four methyl groups were observed at δ 0.77, 0.95, 1.13 and 1.31, indicating the loss of an isopropanol unit. Thus, compound **5** has an α-hydroxy ketone group in its side chain, C-24 and C-25. Methylation of **8** with diazomethane afforded a methyl ester (**9**), $C_{32}H_{48}O_8$ which gave an acetate (**10**), mp 158—159 °C, $C_{34}H_{50}O_9$, after acetylation. In the IR spectrum, **10** showed no absorption due to hydroxyl group. In its ^{1}H-NMR spectrum, signals due to a methoxyl group at δ 3.66 and acetoxyl groups at δ 2.03 (6H, s), and 2.05 (3H, s) were observed. The signal due to H-3 showed a downfield shift from δ 3.26 in **9** to δ 4.55 in **10**.

On a Dreiding model of **2**, if the configuration of C-20 is *S*, C-24 should also be *S*, and if C-20 is *R*, C-24 should be *R*. The determination of the configuration was made by analysis of the nuclear Overhauser effect spectroscopy (NOESY) spectrum of **2** (Fig. 1). The NOE was detected between H-18/Me-26, H-17/Me-21, OAc-16/H-22/H-23, H-15/H-16/H-17, H-12/Me-28 and H-16/Me-28. Thus, the configurations at C-20 and C-24 must be *S*, and H-15 and H-16 are both α-oriented. The stereochemistry of acetoxyl groups at C-15 and 16 was also supported by the comparison of the observed ^{1}H–^{1}H coupling constants ($J_{15,16}=9.0$, $J_{16,17}=11.5$ Hz) with those calculated by Karplus equation$^{5)}$ ($J_{15,16}=8.2$, $J_{16,17}=7.4$ Hz). Accordingly, beesioside I was established as (20*S*,24*S*)-15β,16β-diacetoxy-18,24;20,24-diepoxy-9,19-cyclolanostane-3β,25-diol 3-*O*-β-D-xylopyranoside.

Experimental

General Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Instruments (IR, NMR and optical rotatory dispersion (ORD) spectrum) used in this work were the same as those described in the preceding paper.$^{2b)}$ ^{1}H–^{1}H, ^{1}H–^{13}C COSY and NOESY NMR spectra were recorded with a JEOL JNM GX-400 spectrometer with tetramethylsilane (TMS) as an internal standard. Plant material, extraction and isolation procedures were described earlier.$^{1)}$

Beesioside I (1) Colorless needles, mp 264—266 °C ($CHCl_3$–MeOH, 1 : 1), ORD (c=0.52, $CHCl_3$–MeOH, 1 : 1) $[\alpha]^{25}$ (nm): −10.7° (589), −11.1° (577), −12.5° (546), −22.0° (435), −35.6° (365). *Anal.* Calcd for $C_{39}H_{60}O_{12}\cdot H_2O$: C, 63.39; H, 8.46. Found: C, 63.40; H, 8.34. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3600—3200, 1738, 1238, 1038. ^{1}H-NMR (pyridine-d_5) δ: 2.11 (6H, s, $OCOCH_3$). For ^{13}C-NMR, see Table I.

Enzymatic Hydrolysis of 1 Compound **1** (17 mg) in EtOH (6 ml) was treated with molsin (*Aspergillus saitoi*) (17 mg) in H_2O (6 ml) and 0.2 M Na_2HPO_4–0.1 M citric acid buffer (pH 4.0) (12 ml) and the total mixture was incubated at 37 °C for 14 h. Usual work-up afforded **2** (12 mg), colorless needles, mp 188—190 °C (MeOH). MS *m/z*: 588 $[M]^+$, 570, 528, 468. HR-MS *m/z*: 588.3689 (Calcd 588.3663 for $C_{34}H_{52}O_8$), 528.3428 (Calcd 528.3448 for $C_{32}H_{48}O_6$). IR $\nu_{\max}^{\mathrm{CCl_4}}$ cm^{-1}: 3600, 1740, 1235, 1038. ORD (c=0.91, $CHCl_3$–MEOH, 1 : 1) $[\alpha]^{25}$ (nm): −0.1° (589), −0.2° (577), −0.4° (546), −1.4° (435), −3.3° (365). For ^{1}H- and ^{13}C-NMR, see Tables I and II. Xylose was identified by TLC as the sugar in the water soluble part of the hydrolysis products.

Acetylation of 2 Compound **2** (10 mg) in pyridine (0.1 ml) was acetylated with Ac_2O (0.3 ml) to give **3** (6 mg), colorless needles, mp 214—215 °C (MeOH). *Anal.* Calcd for $C_{36}H_{54}O_9\cdot 3/2H_2O$: C, 65.73; H, 8.73. Found: C, 65.93; H, 8.46. MS *m/z*: 630 $[M]^+$, 615, 612, 570, 510, 450. ^{1}H-NMR ($CDCl_3$) δ: 0.42, 0.66 (each 1H, d, J=4.4 Hz), 0.84, 0.86, 1.13 (3H each, s), 1.22 (6H, s), 1.29 (3H, s), 1.99, 2.02, 2.05 (3H each, s, $OCOCH_3$), 2.49 (1H, d, J=11.0 Hz, H-17), 4.31, 4.53 (1H each, d, J=12.7 Hz, H_2-18), 4.53 (1H, m, H-3), 5.28 (1H, d, J=8.5 Hz, H-15), 5.56 (1H, dd, J=8.5, 11.0 Hz, H-16). Irradiation at δ 5.56 ppm collapsed two doublets at δ 2.49 and 5.28 ppm into two singlets.

Alkaline Treatment of 2 Compound **2** (5 mg) was treated with 2.5% KOH in EtOH (2 ml) at 80 °C for 1.5 h. Usual work-up afforded **4** (4 mg), mp 159—160 °C ($CHCl_3$). MS *m/z*: 504 $[M]^+$, 486, 465, 450. ^{1}H-NMR ($CDCl_3$) δ: 2.34 (1H, d, J=11.0 Hz, H-17), 3.34 (1H, m, H-3), 3.86 (1H, d, J=8.5 Hz, H-15), 4.10 (1H, dd, J=8.5, 11.0 Hz, H-16), 4.17, 4.48 (1H each, d, J=13.0 Hz, H_2-18). Irradiation at δ 4.10 ppm collapsed two doublets at δ 2.34 and 3.86 ppm into two singlets.

Treatment of 2 with *p*-Toluenesulfonic Acid Compound **2** (20 mg) in abs. MeOH (5 ml) was refluxed with *p*-toluenesulfonic acid (3 mg) for 40 min. Usual work-up afforded **5** (16 mg), colorless needles, mp 134—136 °C (hexane), ORD (c=0.4, MeOH) $[\alpha]^{25}$ (nm): −1.2° (589), −1.69° (577), −2.42° (546), −4.85° (435), −8.49° (365). MS *m/z*: 588 $[M]^+$, 570, 473. HR-MS *m/z*: 588.3670 (Calcd 588.3662 for $C_{34}H_{52}O_8$), 473.2864 (Calcd 473.2901 for $C_{28}H_{41}O_6$). IR $\nu_{\max}^{\mathrm{CCl_4}}$ cm^{-1}: 3600, 1738, 1703, 1235, 1040. ^{1}H-NMR ($CDCl_3$) δ: 0.78, 0.95, 1.13, 1.29 (3H each, all s, Me × 4), 1.38 (6H, s, Me × 2), 2.04 (6H, s, $OCOCH_3$ × 2), 2.27 (1H, d, J=10 Hz, H-17), 3.30 (1H, m, H-3), 3.95 (2H, s, H_2-18), 5.24 (1H, t, J=10 Hz, H-16), 5.55 (1H, d, J=10 Hz, H-15).

Alkaline treatment (5% NaOMe in abs. MeOH (2 ml)) followed by acetylation (Ac_2O–pyridine, 1 : 2, 0.2 ml) of **5** (10 mg) gave **6** (8 mg), mp 161—162 °C and **7** (trace). **6**: MS *m/z* 630 $[M]^+$, 615, 612, 570. ^{1}H-NMR ($CDCl_3$) δ: 2.04 (6H, s, $OCOCH_3$), 2.05 (3H, s, $OCOCH_3$), 4.15 (1H, m, H-3). **7**: MS *m/z*: 672 $[M]^+$, 612 $[M-AcOH]^+$. HR-MS *m/z*: 672.3863 (Calcd 672.3871 for $C_{38}H_{56}O_{10}$). ^{1}H-NMR ($CDCl_3$): 2.03 (6H, s, $OCOCH_3$ × 2), 2.05, 2.08 (3H each, s, $OCOCH_3$), 4.55 (1H, m, H-3). Further acetylation of **6** gave **7**. Acid hydrolysis (5% H_2SO_4) followed by acetylation of **1** (2 mg) with Ac_2O–pyridine (1 : 2, 0.5 ml) gave **6** (1 mg) and **7** (trace).

Treatment of 5 with $NaIO_4$ and Cyclohexylamine Compound **5** (3 mg) in MeOH (2 ml) was treated with 0.1 N $NaIO_4$ (1 ml) and cyclohexylamine (1 ml) for 1 h. Usual work-up furnished the acidic product (**8**), colorless needles (2 mg), mp 240—242 °C. MS *m/z*: 546 $[M]^+$, 528, 486, 473. HR-MS *m/z*: 546.3188 (Calcd 546.3191 for $C_{31}H_{46}O_8$). ^{1}H-NMR ($CDCl_3$) δ: 0.77, 0.95, 1.13, 1.31 (3H each, s, Me × 4), 2.03 (6H, s, $OCOCH_3$), 3.28 (1H, m, H-3), 3.98 (2H, brs, H_2-18), 5.24 (1H, t, J=10 Hz, H-16), 5.55 (1H, d, J=10 Hz, H-15).

Methylation of 8 Compound **8** (3 mg) was methylated with ethereal CH_2N_2 to give **9** (3 mg), amorphous powder. MS *m/z*: 560 $[M]^+$, 545, 542, 500, 473. HR-MS *m/z*: 560.3299 (Calcd 560.3347, for $C_{32}H_{48}O_8$), 542.3234 (Calcd 542.3242 for $C_{32}H_{46}O_7$). ^{1}H-NMR ($CDCl_3$) δ: 0.77, 0.94, 1.13, 1.28 (3H each, s, Me × 4), 2.03 (6H, s), 2.27 (1H, d, J=10 Hz, H-17), 3.26 (1H, m, H-3), 3.66 (3H, s, OCH_3), 3.96 (2H, s, H_2-18), 5.25

(1H, t, $J=10$ Hz, H-16), 5.55 (1H, d, $J=10$ Hz, H-15). Irradiation at δ 5.25 ppm collapsed two doublets at δ 2.27 and 5.55 ppm into two singlets.

Acetylation of 9 Compound **9** (2 mg) in pyridine (0.4 ml) was acetylated with Ac_2O (0.2 ml) to give **10** (2 mg), colorless needles, mp 158—160 °C (MeOH). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1735, 1239. MS m/z: 602 $[M]^+$, 542, 473. HR-MS m/z: 602.3491 (Calcd 602.3455, for $C_{34}H_{50}O_9$). ^{1}H-NMR ($CDCl_3$) δ: 0.83, 0.85, 1.13, 1.28 (3H each, s, Me × 4), 2.03 (6H, s, $OCOCH_3$), 2.05 (3H, s, $OCOCH_3$), 2.26 (1H, d, $J=10$ Hz, H-17), 3.66 (3H, s, OCH_3), 3.96 (2H, s, H_2-18), 4.55 (1H, m, H-3), 5.25 (1H, t, $J=10$ Hz, H-16), 5.54 (1H, d, $J=10$ Hz, H-15).

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