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Studies on the Constituents of *Beesia calthaefolia* and *Souliea vaginata*. II. Beesioside II, a Cyclolanostanol Xyloside from Rhizomes of *Beesia calthaefolia*[1,2]

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A new triterpenol xyloside, beesioside II (**2**), mp 149—151 °C, $[\alpha]_D$ +9.3 °, $C_{39}H_{62}O_{12}\cdot 0.5H_2O$, from the dried rhizomes of *Beesia calthaefolia* MAXIM. (Ranunculaceae), was identified as (20*S*,24*R*)-16β,18-diacetoxy-20,24-epoxy-9,19-cyclolanostane-3β,15α,25-triol 3-*O*-β-D-xylopyranoside on the basis of chemical and physicochemical evidence. The structure of **2** was confirmed by an X-ray structure analysis of a derivative.

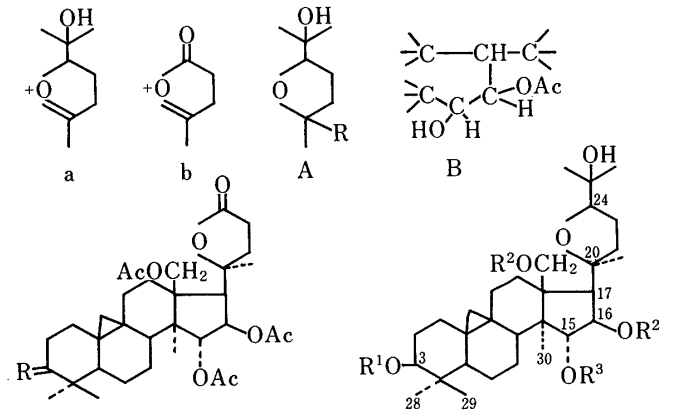
Keywords——*Beesia calthaefolia*; Ranunculaceae; beesioside II; X-ray structure analysis; ^{13}C-NMR; 9,19-cyclolanostanol xyloside; triterpenol glycoside

In the preceding paper,[2] we reported the isolation of four new triterpenol xylosides from the rhizomes of *Beesia calthaefolia* MAXIM. and two of the four from *Souliea vaginata* (MAXIM.) FRANCH. (both Ranunculaceae). The structure of beesioside III (**1**), one of the new triterpenol xylosides, has been determined as 15α-acetoxy-20ξ_1,24ξ_2-epoxy-9,19-cyclolanostane-3β,12β,16β,25-tetraol 3-*O*-β-D-xylopyranoside. This paper deals with the structure elucidation of a minor triterpenol xyloside, beesioside II (**2**) on the basis of chemical and physicochemical evidence. In order to complete elucidation of the structure, including the side chain stereochemistry of **2**, X-ray structure analysis of the diacetylated aglycone (**4**) has been carried out.

Beesioside II (**2**), mp 149—151 °C, $C_{39}H_{62}O_{12}\cdot 0.5H_2O$ showed strong absorption bands of hydroxyl groups (3450 (br), 1050 (br) cm^{-1}) and carbonyl groups (1745, 1730 cm^{-1}) in its infrared (IR) spectrum. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **2** showed two acetoxyl signals at δ 21.5, 21.7, 170.1 and 171.4 ppm.

On enzymatic hydrolysis with molsin, **2** gave an aglycone (**3**), $C_{34}H_{54}O_8$, and xylose. The mass spectrum (MS) of **3** gave a fragment at *m/z* 143 as the base peak, which is assignable to a substituted tetrahydrofuran ion (a) as seen in beesioside III (**1**)[2] and other ocotillone-type triterpenes.[3] The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **3** showed a double doublet at δ 3.71 ppm (J=8.0 and 6.6 Hz) which was assignable to H-24 in the partial structure A (Chart 1).

Comparisons of the ^{13}C-NMR data of **2** with those of **1** disclosed the presence of the side chain A in **2**. Furthermore, detailed ^{1}H- and ^{13}C-NMR examinations of **3** revealed that **3** possessed six tertiary methyls and a cyclopropane ring, along with the side chain A and two secondary hydroxyl groups. An AB quartet centered at δ 4.54 ppm (2H, J=12.2 Hz) indicated



a b A B

6 : R=OAc, H
7 : R=O

	R^1	R^2	R^3
2 :	β-xyl	$COCH_3$	H
3 :	H	$COCH_3$	H
4 :	$COCH_3$	$COCH_3$	$COCH_3$
9 :	H	H	H

Chart 1

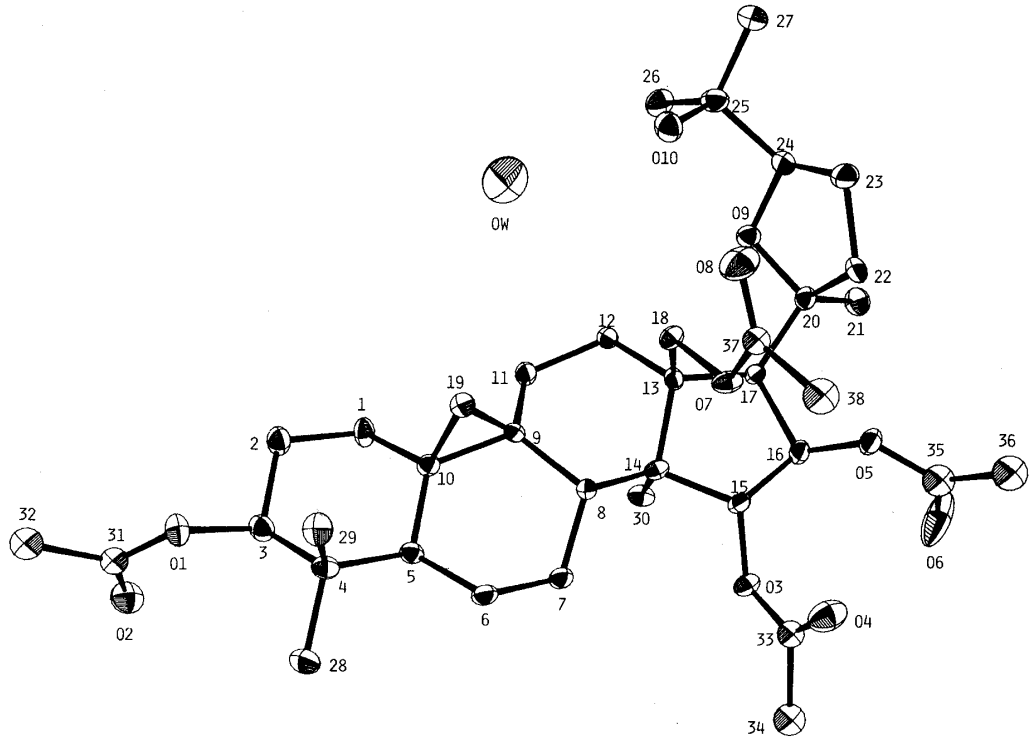


Fig. 1. Molecular Structure of the Tetraacetate (**4**)

the presence of an acetyloxymethyl group. The ^{13}C-NMR data of **3**, in comparison with those[2] of the genin (**5**) of beesioside III (**1**) and beesioside III (**1**), led us to conclude that the genin (**3**) of beesioside II (**2**) is a 9,19-cyclolanostane triterpene (Table I).

Spin decoupling experiments were undertaken to assign ABX signals of **3**. Irradiation at δ 5.05 ppm (H-16) (B part of ABX) collapsed two doublets at δ 2.64 ppm (H-17) (X part of ABX) and δ 4.15 ppm (H-15) (A part of ABX) into two singlets. Therefore one of the two acetoxyl groups in **3** is located at C-16, one of the two secondary hydroxyl groups at C-15, and the side chain A, at C-17. These findings indicate that **3** has a partial structure B.

Acetylation of **3** afforded a diacetylated genin, tetraacetate (**4**), mp 192—193 °C,

TABLE I. ^{13}C-NMR Data for Beesioside III (**1**), Beesioside II (**2**), Genin (**3**) and Deacetylated Genin (**9**)

Carbon No.	**1**	**2**	**3**	**9**
1	32.3	32.4	32.4	32.5
2	29.9	30.0	31.0	31.6
3	88.4	88.3	77.7	77.9
4	41.2	41.3	40.9	41.0
5	48.3	47.7	47.3	47.6
6	21.1	21.0	21.0	21.3
7	26.1	26.3	25.9	26.5
8	48.1	48.6	48.4	48.4
9	19.6	20.2	20.0	20.3
10	25.9	26.9	27.0	26.8
11	35.3	25.8	25.9	24.5
12	73.0	30.2	30.1	30.6
13	48.3	49.8	49.6	53.0
14	51.7	49.8	49.6	49.6
15	91.3	83.7	83.5	86.8
16	77.9	83.7	83.3	82.4
17	48.9	54.3	54.2	53.3
18	20.2	67.2	67.2	65.8
19	29.4	31.2	31.0	31.1
20	85.8	84.5	84.4	86.2
21	28.8[a)]	27.7[a)]	27.4[a)]	28.4[a)]
22	36.5	36.8	36.7	36.9
23	26.3	26.1	25.9	29.5
24	83.1	83.7	83.5	85.2
25	69.9	70.3	70.2	70.6
26	27.5	26.6[a)]	26.6[a)]	26.3[a)]
27	27.5	27.5[a)]	27.2[a)]	26.3[a)]
28	25.7[a)]	26.6[a)]	26.5[a)]	26.1[a)]
29	14.1	15.4	14.7	14.7
30	15.3	13.8	13.6	13.6
OAc	171.8	170.1	170.2	
OAc		171.4	171.3	
OAc	21.3	21.5	21.3	
OAc		21.7	21.5	
1′	107.3	107.3		
2′	75.3	75.3		
3′	78.3	78.3		
4′	71.0	71.0		
5′	66.9	66.9		

a) Values in any vertical column may be reversed although those given here are preferred.

$C_{38}H_{58}O_{10}$, which also has a hydroxyl group. The ^{1}H-NMR spectrum of **4** also showed the ABX system at δ 2.62 (H-17), 5.65 (H-16) and 5.71 (H-15) ppm. Jones oxidation of **4** afforded a tetraacetyltrisnorlactone (**6**), $C_{35}H_{50}O_{10}$ and a triacetyltrisnorketolactone (**7**), $C_{33}H_{44}O_9$, which gave a peak at *m/z* 99 ($C_5H_7O_2$) indicative of ion b in their MS. Facile loss of the hydroxyisopropyl group producing a γ-lactone has been reported in other triterpenes with this type of side chain A.[4)] The loss of signals due to two methyl groups and the H-24 proton was confirmed by the ^{1}H-NMR spectrum. The occurrence of a five-membered lactone was also indicated by the IR spectrum (1778 cm^{-1}). This evidence strongly suggested that the aglycone (**3**) has a side chain A.

In the ^{1}H-NMR spectrum the coupling constants ($J_{15,16}$ = 4.2 Hz and $J_{16,17}$ = 9.5 Hz) of

TABLE II. Bond Lengths (Å) with Estimated Standard Deviations in Parentheses

C(1)–C(2)	1.536 (18)	C(1)–C(10)	1.548 (17)
C(2)–C(3)	1.530 (18)	C(3)–C(4)	1.562 (17)
C(4)–C(5)	1.558 (16)	C(4)–C(28)	1.544 (18)
C(4)–C(29)	1.527 (19)	C(5)–C(6)	1.509 (16)
C(5)–C(10)	1.512 (16)	C(6)–C(7)	1.550 (17)
C(7)–C(8)	1.546 (16)	C(8)–C(9)	1.553 (15)
C(8)–C(14)	1.545 (16)	C(9)–C(10)	1.531 (16)
C(9)–C(11)	1.542 (17)	C(9)–C(19)	1.513 (16)
C(10)–C(19)	1.521 (16)	C(11)–C(12)	1.544 (16)
C(12)–C(13)	1.536 (16)	C(13)–C(14)	1.575 (15)
C(13)–C(17)	1.553 (15)	C(13)–C(18)	1.583 (16)
C(14)–C(15)	1.556 (16)	C(14)–C(30)	1.519 (17)
C(15)–C(16)	1.533 (16)	C(16)–C(17)	1.574 (16)
C(17)–C(20)	1.550 (16)	C(20)–C(21)	1.543 (18)
C(20)–C(22)	1.567 (18)	C(22)–C(23)	1.548 (19)
C(23)–C(24)	1.543 (19)	C(24)–C(25)	1.587 (18)
C(25)–C(26)	1.515 (19)	C(25)–C(27)	1.499 (19)
C(31)–C(32)	1.498 (21)	C(33)–C(34)	1.546 (21)
C(35)–C(36)	1.465 (24)	C(37)–C(38)	1.514 (20)
C(3)–O(1)	1.456 (15)	C(15)–O(3)	1.454 (14)
C(16)–O(5)	1.441 (14)	C(18)–O(7)	1.461 (14)
C(20)–O(9)	1.458 (14)	C(24)–O(9)	1.413 (15)
C(25)–O(10)	1.424 (16)	C(31)–O(1)	1.338 (16)
C(31)–O(2)	1.174 (17)	C(33)–O(3)	1.331 (15)
C(33)–O(4)	1.183 (17)	C(35)–O(5)	1.345 (19)
C(35)–O(6)	1.202 (23)	C(37)–O(7)	1.321 (15)
C(37)–O(8)	1.210 (17)		

H-16 in **3** supported the *cis* relationship of the 16-acetoxyl group and the side chain A. Consequently, the 15α-hydroxyl and 16β-acetoxyl configurations in **3** were substantiated. The signal due to H-3 in **3** was assigned from its chemical shift and coupling patterns.

The position of the acetyloxymethyl group in the xyloside (**2**) was speculative, based on the ^{13}C-NMR spectrum. In **1** and cimigenol xyloside (**8**) a resonance at *ca.* 19.0 ppm was assigned to the C-18 methyl group,[2] but the resonance was not detected in **2**. Alkaline treatment of **3** gave the deacetylated genin (**9**), $C_{30}H_{50}O_6$. The triplet at δ 67.2 ppm was assigned to C-18 of **3**: it is deshielded by 1.4 ppm in the spectrum of **9**.[4]

From the above evidence, the aglycone (**3**) can be characterized as a 9,19-cyclolanostane triterpene possessing 16β,18-diacetoxyl, 3β,15α-dihydroxy groups and the side chain A. There remains the question of the stereochemistries at C-20 and C-24.

The sugar moiety of the xyloside (**2**) was shown to be attached to C-3 of the aglycone (**3**) as β-D-xylopyranose by comparison of the ^{13}C-NMR chemical shifts of the anomeric carbon and C-3 in beesioside III (**1**).[2] Application of Klyne's rule[5] to **2** and **3** also supported the β-D-xylopyranoside structure [molecular rotation difference at 589 nm of **2** and **3**, −102°; methyl β-D-xylopyranoside, M_D −108°; methyl α-D-xylopyranoside, M_D +253°].[6]

In order to determine the exact structure of **3** including the stereochemistry, an X-ray structure analysis of the tetraacetate (**4**) was undertaken. Crystals of **4** monohydrate were grown in an aq. ethanol solution as colorless prisms. The molecular structure of **4** is illustrated in Fig. 1. Bond lengths and angles for **4** are listed in Tables II and III. Based on the O(2)–O(10) and O(10)–O(W) distances (2.86 Å and 2.80 Å), the bond O(2)–H–O(10)–H–O(W) seems to be an internal hydrogen bond. The relevant torsion angles are given in Table IV. The torsion angles H_{17}–C_{17}–C_{16}–H_{16} (*ca.* 15°) and H_{16}–C_{16}–C_{15}–H_{15} (*ca.* −143°) estimated from

TABLE III. Bond Angles (°) with Estimated Standard Deviations in Parentheses

C(2)–C(1)–C(10)	109.6 (1.0)	C(1)–C(2)–C(3)	110.8 (1.1)
C(2)–C(3)–C(4)	114.9 (1.0)	C(2)–C(3)–O(1)	106.2 (1.0)
C(4)–C(3)–O(1)	108.7 (0.9)	C(3)–C(4)–C(5)	105.9 (0.9)
C(3)–C(4)–C(28)	107.5 (1.0)	C(3)–C(4)–C(29)	110.9 (1.0)
C(5)–C(4)–C(28)	110.9 (1.0)	C(5)–C(4)–C(29)	113.1 (1.0)
C(28)–C(4)–C(29)	108.4 (1.0)	C(4)–C(5)–C(6)	113.9 (1.0)
C(4)–C(5)–C(10)	112.4 (0.9)	C(6)–C(5)–C(10)	111.9 (0.9)
C(5)–C(6)–C(7)	109.0 (1.0)	C(6)–C(7)–C(8)	109.4 (0.9)
C(7)–C(8)–C(9)	111.7 (0.9)	C(7)–C(8)–C(14)	113.5 (0.9)
C(9)–C(8)–C(14)	110.4 (0.9)	C(8)–C(9)–C(10)	117.6 (0.9)
C(8)–C(9)–C(11)	118.0 (0.9)	C(8)–C(9)–C(19)	114.8 (0.9)
C(10)–C(9)–C(11)	116.2 (0.9)	C(10)–C(9)–C(19)	59.9 (0.7)
C(11)–C(9)–C(19)	117.5 (0.9)	C(1)–C(10)–C(5)	110.9 (0.9)
C(1)–C(10)–C(9)	118.0 (1.0)	C(1)–C(10)–C(19)	116.0 (0.9)
C(5)–C(10)–C(9)	121.9 (0.9)	C(5)–C(10)–C(19)	121.9 (1.0)
C(9)–C(10)–C(19)	59.5 (0.7)	C(9)–C(11)–C(12)	116.2 (1.0)
C(11)–C(12)–C(13)	114.3 (0.9)	C(12)–C(13)–C(14)	107.8 (0.9)
C(12)–C(13)–C(17)	115.3 (0.9)	C(12)–C(13)–C(18)	103.2 (0.9)
C(14)–C(13)–C(17)	101.9 (0.8)	C(14)–C(13)–C(18)	115.0 (0.9)
C(17)–C(13)–C(18)	113.9 (0.9)	C(8)–C(14)–C(13)	118.1 (0.9)
C(8)–C(14)–C(15)	113.6 (0.9)	C(8)–C(14)–C(30)	112.8 (0.9)
C(13)–C(14)–C(15)	100.2 (0.9)	C(13)–C(14)–C(30)	112.9 (0.9)
C(15)–C(14)–C(30)	108.7 (0.9)	C(14)–C(15)–C(16)	105.2 (0.9)
C(14)–C(15)–O(3)	111.0 (0.9)	C(16)–C(15)–O(3)	111.0 (0.9)
C(15)–C(16)–C(17)	105.3 (0.9)	C(15)–C(16)–O(5)	107.7 (0.9)
C(17)–C(16)–O(5)	112.9 (0.9)	C(13)–C(17)–C(16)	105.7 (0.9)
C(13)–C(17)–C(20)	121.6 (0.9)	C(16)–C(17)–C(20)	116.4 (0.9)
C(13)–C(18)–O(7)	110.7 (0.9)	C(9)–C(19)–C(10)	60.6 (0.8)
C(17)–C(20)–C(21)	109.1 (1.0)	C(17)–C(20)–C(22)	120.1 (1.0)
C(17)–C(20)–O(9)	104.3 (0.9)	C(21)–C(20)–C(22)	110.0 (1.0)
C(21)–C(20)–O(9)	108.7 (0.9)	C(22)–C(20)–O(9)	103.9 (0.9)
C(20)–C(22)–C(23)	105.0 (1.0)	C(22)–C(23)–C(24)	104.1 (1.1)
C(23)–C(24)–C(25)	113.9 (1.1)	C(23)–C(24)–O(9)	107.5 (1.0)
C(25)–C(24)–O(9)	109.0 (1.0)	C(24)–C(25)–C(26)	108.2 (1.1)
C(24)–C(25)–C(27)	108.4 (1.1)	C(24)–C(25)–O(10)	108.8 (1.0)
C(26)–C(25)–C(27)	112.9 (1.1)	C(26)–C(25)–O(10)	106.7 (1.0)
C(27)–C(25)–O(10)	111.7 (1.1)	C(32)–C(31)–O(1)	111.8 (1.1)
C(32)–C(31)–O(2)	126.0 (1.3)	O(1)–C(31)–O(2)	122.1 (1.2)
C(34)–C(33)–O(3)	110.6 (1.1)	C(34)–C(33)–O(4)	124.4 (1.3)
O(3)–C(33)–O(4)	125.0 (1.2)	C(36)–C(35)–O(5)	113.4 (1.4)
C(36)–C(35)–O(6)	125.6 (1.7)	O(5)–C(35)–O(6)	120.8 (1.5)
C(38)–C(37)–O(7)	113.2 (1.1)	C(38)–C(37)–O(8)	124.1 (1.2)
O(7)–C(37)–O(8)	122.5 (1.2)	C(3)–O(1)–C(31)	120.8 (1.0)
C(15)–O(3)–C(33)	116.3 (0.9)	C(16)–O(5)–C(35)	118.7 (1.0)
C(18)–O(7)–C(37)	118.0 (0.9)	C(20)–O(9)–C(24)	108.6 (0.8)

TABLE IV. Selected Torsion Angles (°), with Estimated Standard Deviations in Parentheses

C(14)–C(15)–C(16)–C(17)	−23.1 (1.0)
C(14)–C(15)–C(16)–O(5)	−143.8 (0.8)
O(3)–C(15)–C(16)–C(17)	−143.3 (0.8)
O(3)–C(15)–C(16)–O(5)	96.1 (0.9)
C(15)–C(16)–C(17)–C(13)	−5.9 (1.0)
C(15)–C(16)–C(17)–C(20)	−144.3 (0.9)
O(5)–C(16)–C(17)–C(13)	111.3 (0.9)
O(5)–C(16)–C(17)–C(20)	−27.1 (1.2)

TABLE V. Atomic Positional Parameters with Estimated Standard Deviations in Parentheses and Equivalent Isotropic Thermal Parameters

Atom	$x \times 10^4$	$y \times 10^4$	$z \times 10^4$	B_{eq} (Å^2)
C(1)	7636 (10)	4588 (4)	6440 (12)	3.68
C(2)	7757 (10)	4223 (4)	7025 (13)	4.20
C(3)	7020 (10)	3831 (4)	7332 (11)	3.82
C(4)	5988 (9)	3920 (3)	8263 (11)	3.15
C(5)	5310 (9)	4292 (3)	7602 (11)	2.84
C(6)	4209 (9)	4398 (3)	8292 (11)	3.38
C(7)	3593 (9)	4758 (3)	7511 (12)	3.36
C(8)	4240 (8)	5186 (3)	7690 (11)	2.69
C(9)	5517 (8)	5139 (3)	7338 (11)	2.70
C(10)	6031 (8)	4687 (3)	7379 (11)	2.82
C(11)	6021 (9)	5449 (3)	6305 (12)	3.31
C(12)	5565 (9)	5913 (3)	6331 (11)	2.97
C(13)	4475 (8)	5972 (3)	7144 (10)	2.73
C(14)	3709 (8)	5564 (3)	6918 (10)	2.84
C(15)	2569 (8)	5726 (3)	7514 (11)	3.12
C(16)	2441 (9)	6188 (4)	7015 (11)	3.34
C(17)	3682 (8)	6340 (3)	6677 (10)	2.72
C(18)	4934 (9)	6029 (3)	8606 (10)	2.88
C(19)	6271 (9)	5007 (3)	8482 (11)	3.06
C(20)	3960 (9)	6818 (3)	6964 (11)	3.06
C(21)	3339 (11)	7103 (4)	5948 (12)	4.16
C(22)	3798 (10)	7004 (3)	8392 (12)	3.58
C(23)	4850 (10)	7291 (4)	8627 (13)	4.70
C(24)	5585 (10)	7223 (3)	7380 (12)	3.75
C(25)	6898 (10)	7166 (4)	7682 (12)	4.00
C(26)	7494 (10)	7054 (4)	6399 (12)	4.41
C(27)	7331 (11)	7573 (4)	8292 (15)	5.32
C(28)	5269 (11)	3507 (4)	8322 (13)	4.37
C(29)	6388 (11)	4028 (4)	9662 (12)	4.15
C(30)	3526 (10)	5462 (4)	5463 (10)	3.31
C(31)	8103 (10)	3167 (4)	7326 (12)	3.91
C(32)	9025 (12)	2936 (4)	8058 (14)	5.26
C(33)	787 (10)	5404 (4)	7946 (12)	3.91
C(34)	−124 (12)	5098 (4)	7378 (15)	5.50
C(35)	838 (13)	6560 (5)	7919 (15)	5.62
C(36)	371 (13)	6765 (5)	9105 (16)	6.34
C(37)	4164 (10)	6184 (4)	10738 (11)	3.55
C(38)	3132 (12)	6155 (4)	11626 (15)	5.68
O(1)	7765 (7)	3520 (2)	7968 (8)	4.26
O(2)	7679 (8)	3055 (3)	6333 (9)	5.49
O(3)	1624 (6)	5461 (2)	7079 (7)	3.49
O(4)	749 (8)	5560 (3)	9012 (10)	6.20
O(5)	1925 (6)	6436 (2)	8057 (8)	3.77
O(6)	318 (9)	6481 (5)	6925 (13)	11.94
O(7)	4000 (6)	6013 (2)	9556 (7)	3.37
O(8)	5030 (8)	6370 (3)	11030 (10)	6.39
O(9)	5179 (6)	6851 (2)	6736 (7)	3.27
O(10)	7042 (7)	6808 (3)	8544 (8)	4.53
O(w)	8082 (12)	6001 (5)	8342 (17)	12.39

Table IV are in good agreement with the values calculated from the coupling constants in the ^{1}H-NMR spectrum of **4**. The absolute stereochemistries at C-20 and C-24 were established as *S* and *R*, respectively (Chart 1). Accordingly, the structure of beesioside II (**2**) was established

as (20*S*,24*R*)-16β,18-diacetoxy-20,24-epoxy-9,19-cyclolanostane-3β,15α,25-triol 3-*O*-β-D-xylopyranoside.

Isolation and structure determination of other minor triterpenol xylosides of this herb are in progress.

Experimental

All melting points were measured on a Shimadzu micro melting point determination apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-181 automatic polarimeter in a 1 dm tube. ^{1}H- and ^{13}C-NMR spectra were recorded with a JEOL JNM FX-100 spectrometer at 100 and 25 MHz, respectively. ^{1}H- and ^{13}C-NMR spectra were taken in $CDCl_3$ and C_5D_5N, respectively. Tetramethylsilane was used as the internal standard. Chemical shifts are given on the δ scale (ppm). The following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Coupling constants (*J* values) are given in Hz. Mass spectra were recorded with a JEOL JMS-D300 at 70 eV. For thin layer chromatography (TLC), pre-coated silica gel plates (Silica gel 60 F-254, Merck) were used, and detection was carried out by spraying 10% H_2SO_4 followed by heating.

Isolation of Beesioside II (2)——In a previous paper,[2] we described the isolation of **2** (30 mg) from the dried rhizomes of *Beesia calthaefolia* (39 g). Samples for analysis were obtained by high performance liquid chromatography (HPLC) [Shimadzu LC-3A instrument; Zorbax ODS (0.25 μ, 4.6 mm × 25 cm) column; mobile phase, MeOH–H_2O (85 : 15); temperature, room temperature; flow rate, 1.2 ml/min; monitored with a differential refractometer; sensitivity, 16×10^{-5} RIFS.]

Beesioside II (**2**): amorphous powder, mp 149—151 °C, $[\alpha]_D^{15}$ +9.3° (*c* = 0.8, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3450, 1745, 1730, 1260, 1050. *Anal.* Calcd for $C_{39}H_{62}O_{12} \cdot 0.5H_2O$: C, 64.00, H, 8.61. Found: C, 63.66; H, 8.75.

Enzymatic Hydrolysis of Beesioside II (2)——Compound **2** (50 mg) in EtOH (5 ml) was treated with molsin (from *Aspergillus saitoi*) (50 mg) in H_2O (5 ml) and 0.2 M Na_2HPO_4–0.1 M citric acid buffer (pH 4.0) (10 ml), and the total mixture was incubated at 37 °C for 17 h. After usual work-up, the crude product (30 mg) was purified by column chromatography (silica gel, benzene–EtOAc = 1 : 3), to give the aglycone (**3**, 13 mg), amorphous powder, $[\alpha]_D^{23}$ +28.7° (*c* = 0.7, MeOH). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3650, 3505, 1738, 1719, 1250, 1230, 1058, 1035. MS *m/z*: 590.3778 (M^+) (Calcd for $C_{34}H_{54}O_8$, 590.3818), 572 ($M^+ - H_2O$), 554 ($M^+ - 2H_2O$), 530.3603 ($M^+ - CH_3COOH$) (Calcd for $C_{32}H_{50}O_6$, 530.3606), 470.3377 ($M^+ - 2CH_3COOH$) (Calcd for $C_{30}H_{46}O_4$, 470.3394), 143.1045 (ion a) (Calcd for $C_8H_{15}O_2$ 143.1070), 125.0941 (Calcd for $C_8H_{13}O$, 125.0966). ^{1}H-NMR: 0.34, 0.62 (1H each, d, *J* = 4.4 Hz, cyclopropane methylene), 0.79, 0.96 (3H each, s, $2CH_3$), 1.11 (6H, s, $2CH_3$), 1.19, 1.27 (3H each, s, $2CH_3$), 2.09, 2.19 (3H each, s, $2COCH_3$), 2.64 (1H, d, *J* = 9.5 Hz, H-17), 3.3 (1H, m, H-3), 3.70 (1H, m, OH), 3.71 (1H, dd, *J* = 8.0 and 6.6 Hz, H-24), 4.15 (1H, d, *J* = 4.2 Hz, H-15), 4.29, 4.79 (1H each, d, *J* = 12.2 Hz, $C\underline{H}_2OCOCH_3$), 5.05 (1H, dd, *J* = 9.5 and 4.2 Hz, H-16). ^{13}C-NMR: Table I.

Acetylation of the Aglycone (3)——Compound **3** (9 mg) was acetylated with Ac_2O (0.2 ml) in pyridine (0.5 ml) at room temperature overnight. After usual work-up, the crude product was purified by column chromatography (silica gel, benzene–EtOAc = 2 : 1), to give the tetraacetate (**4**, 7 mg), colorless needles (from EtOH), mp 192—193 °C,[7] $[\alpha]_D^{19}$ +39.6° (*c* = 1.0, MeOH). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3510, 1738, 1238, 1040. MS *m/z*: 674 (M^+), 659 ($M^+ - CH_3$), 656 ($M^+ - H_2O$), 614, 554, 494, 434. ^{1}H-NMR: 0.43, 0.67 (1H each, d, *J* = 4.6 Hz), 0.83, 0.87, 1.10, 1.16, 1.18, 1.26 (3H each, s, $6CH_3$), 1.99, 2.05, 2.10, 2.21 (3H each, s, $4COCH_3$), 2.62 (1H, d, *J* = 8.8 Hz, H-17), 3.68 (1H, t, *J* = 8 Hz, H-24), 4.25 (1H, d, *J* = 12.2 Hz, H-18), 4.56 (1H, m, H-3), 4.84 (1H, d, *J* = 12.2 Hz, H-18), 5.65 (1H, dd, *J* = 5.1 and 8.8 Hz, H-16), 5.71 (1H, d, *J* = 5.1 Hz, H-15). *Anal.* Calcd for $C_{38}H_{58}O_{10}$: C, 67.63; H, 8.66. Found: C, 67.45; H, 8.93.

Jones Oxidation of the Tetraacetate (4)——Compound **4** (13 mg) in Me_2CO was oxidized with Jones reagent (0.3 ml) at room temperature for 14 h. After usual work-up, the product was purified by column chromatography (silica gel, benzene–EtOAc = 3 : 1), to furnish the tetraacetyltrisnorlactone (**6**, 5 mg), amorphous powder, and the triacetyltrisnorketolactone (**7**, 2 mg), colorless needles (from MeOH), mp 248—250 °C. Compound **6**: IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1778, 1740, 1242. MS *m/z*: 630.3388 (M^+) (Calcd for $C_{35}H_{50}O_{10}$, 630.3403), 570.3169 ($M^+ - CH_3COOH$) (Calcd for $C_{33}H_{46}O_8$, 570.3191), 510.2991 ($M^+ - 2CH_3COOH$) (Calcd for $C_{31}H_{42}O_6$, 510.2981), 99 (ion b). ^{1}H-NMR: 0.84, 0.87, 1.16, 1.43 (3H each, s, $4CH_3$), 2.00, 2.03, 2.05, 2.21 (3H each, s, $4COCH_3$), 2.58 (1H, d, *J* = 9.1 Hz, H-17), 4.42 (1H, d, *J* = 12.2 Hz, H-18), 4.60 (1H, m, H-3), 4.73 (1H, d, *J* = 12.2 Hz, H-18), 5.60 (1H, dd, *J* = 9.1 and 4.9 Hz, H-16), 5.71 (1H, d, *J* = 4.9 Hz, H-15). Compound **7**: IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1779, 1745, 1708, 1240. MS *m/z*: 586.3125 (M^+) (Calcd for $C_{33}H_{46}O_9$, 586.3140), 568.3058 ($M^+ - H_2O$) (Calcd for $C_{33}H_{44}O_8$, 568.3037), 99 (ion b). ^{1}H-NMR: 1.04, 1.08, 1.18, 1.45 (3H each, s, $4CH_3$), 2.01, 2.04, 2.23 (3H each, s, $3COCH_3$), 2.60 (1H, d, *J* = 9.1 Hz, H-17), 4.45, 4.63 (1H each, d, *J* = 12.2 Hz, 2H-18), 5.62 (1H, dd, *J* = 9.1 and 4.9 Hz, H-16), 5.73 (1H, d, *J* = 4.9 Hz, H-15).

Alkaline Treatment of the Aglycone (3)——A solution of **3** (7 mg) in 0.1% NaOMe–MeOH (2 ml) was left standing at room temperature overnight. After work-up in the usual manner, the product was purified by column chromatography (silica gel, benzene–EtOAc = 1 : 3), to furnish **9** (3 mg), amorphous powder. MS *m/z*: 506.3600 (M^+) (Calcd for $C_{30}H_{50}O_6$, 506.3605), 488, 470, 452, 143 (base peak). ^{1}H-NMR: 0.81, 0.97, 1.00, 1.12 (3H each, s, $4CH_3$),

1.26 (6H, s, $2CH_3$), 2.32 (1H, d, $J=9$ Hz, H-17), 3.28 (1H, m, H-3), 3.90 (1H, dd, $J=8.0$ and 6.6 Hz, H-24), 4.03 (1H, br s, H-15), 4.16 (1H, br d, $J=9$ Hz, H-16), 4.24 (2H, s, 2H-18).

X-Ray Structure Analysis of the Tetraacetate (4) Monohydrate——The compound (**4**) was grown from aq. EtOH to yield **4** monohydrate as colorless prisms, mp 191—193 °C. *Anal.* Calcd for $C_{38}H_{58}O_{10}\cdot 0.5H_2O$: C, 66.74; H, 8.70. Found: C, 66.72; H, 8.82.

Crystal Data: $P2_12_12_1$ (orthorhombic), $a=11.779$ (4), $b=31.151$ (2), $c=10.108$ (1) Å; $V=3708.9$ (8) Å^3; $Z=4$; $D_c=1.24\,g\cdot cm^{-3}$; $F(000)=1504$. The diffraction intensities were collected from a crystal of dimensions $0.5\times0.5\times0.2$ mm on a Rigaku AFC-5 FOS four-circle diffractometer using MoK_α radiation monochromated by means of a graphite plate. A total of 2434 reflections were measured in a 2θ range of 2—50 ° as above the $3\sigma(F)$ level. These were used in the solution and refinement of the structure.

Determination of the Structure: The structure was solved by the direct method using MULTAN[7] and refined by the block-matrix least-squares method. In the final refinement, anisotropic thermal parameters were used for non-hydrogen atoms. The final R factor without hydrogen atoms was 0.098. The data were not corrected for the effects of absorption. The final atomic parameters are shown in Table V, and bond lengths and angles in Tables II and III.[8]

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References and Notes

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