STUDIES ON THE CONSTITUENTS OF BEESIA CALTHAEFOLIA, AND SOULIEA VAGINATA. III. BEESIOSIDE IV, A CYCLOLANOSTANOL XYLOSIDE FROM THE RHIZOMES OF B. CALTHAEFOLIA AND S. VAGINATA

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Abstract —— A new triterpenol xyloside, beesioside IV, an amorphous powder, $[\alpha]_D$ -11.1°, $C_{37}H_{58}O_{11}\cdot 3/2H_2O$ from the dried rhizomes of Beesia calthaefolia Maxim. and Souliea vaginata (Maxim.) Franch. (both Ranunculaceae) was identified as (20S, 24S)-15α-acetoxy-16β,24;20,24-diepoxy-9,19-cyclolanostane-3β, 12α, 25-triol 3-O-β-D-xylopyranoside on the basis of chemical and physicochemical evidence.

In the previous paper,[1] we reported the isolation of four new triterpenol xylosides from the rhizomes of Beesia calthaefolia Maxim. and two out of the four from Souliea vaginata (Maxim.) Franch. (Ranunculaceae). The structures of Beesiosides II and III have been determined as (20S, 24R)-16β,18-diacetoxy-20,24-epoxy-9,19-cyclolanostane-3β,15α,25-triol 3-O-β-D-xylopyranoside[2] and 15α-acetoxy-20ξ_1,24ξ_2-epoxy-9,19-cyclolanostane-3β,12β,16β,25-tetraol 3-O-β-D-xylopyranoside,[1] respectively. This paper deals with the structure elucidation of beesioside IV isolated from the both plants on the basis of chemical and physicochemical evidence.

Beesioside IV (**1**) is an amorphous powder, $[\alpha]_D$ -11.1°. The result of elemental analysis was consistent with $C_{37}H_{58}O_{11}\cdot 3/2H_2O$. The ir spectrum of **1** showed strong absorption bands of hydroxyl (3400 cm^{-1}) and a carbonyl (1720 cm^{-1}). The ^1H- and ^{13}C-nmr data indicated that **1** is a 9, 19-cyclolanostane triterpenol xyloside having an acetoxyl group and a ketal carbon (Table I).

On enzymatic hydrolysis with molsin, **1** gave an aglycone (**2**), $C_{32}H_{50}O_7$, $[\alpha]_D$+ 1.6° and xylose. The ^1H- and ^{13}C-nmr spectra of **2** showed that **2** possesses a cyclopropane ring, an acetoxy group and seven tertiary methyl groups (Table II, III). The multiplet at δ 3.28 was assigned to H-3 by comparison with those in the ^1H-nmr spectra of beesiosides II and III. The two-dimensional ^1H-^1H correlation spectroscopy (^1H-^1H COSY) of **2** revealed the presence of a partial structure A. The signal due to H_a (H-16, dd, J=7.3, 2.9 Hz) at δ 4.18 connected with the signals due to H_b (H-15, d, J=2.9) at δ 5.23 and H_c (H-17, d, J=7.3) at δ 2.30 . The partial

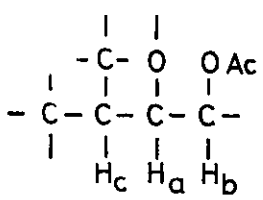
Table I. ^{13}C-Nmr Chemical Shifts of Beesioside IV (**1**) and Monoacetate (**4**) in d_5-Pyridine

Carbon	**1**	**4**	Carbon	**1**	**4**	Carbon	**1**	**4**
1	32.5	32.1	14	50.7	50.9	27	25.1	25.2
2	29.9	27.2	15	86.7	86.8	28	24.5	24.6
3	88.4	79.8	16	79.7	80.4	29	15.3	15.4
4	41.2	39.7	17	44.1	44.2	30	14.7	14.7
5	47.6	47.3	18	20.5	20.6	OAc	170.1	170.5
6	21.1	21.1	19	29.5	30.0		21.4	21.4
7	25.7	26.1	20	82.8	83.0	OAc		170.2
8	48.1	48.2	21	26.2	25.6			21.1
9	19.8	20.2	22	40.2	40.3	1'	107.3	
10	26.2	26.1	23	28.6	28.7	2'	75.2	
11	39.9	39.8	24	110.3	110.5	3'	78.3	
12	72.0	72.2	25	72.0	72.2	4'	70.9	
13	49.7	49.8	26	25.2	25.3	5'	66.9	

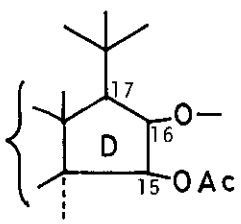
Table II. ^{1}H-Nmr Spectral Data (δ ppm and J/Hz) of Compounds **2-7** in $CDCl_3$

Proton	**2**	**3**	**4**	**5**	**6**	**7**
3	3.28m	3.30m	4.54m	4.58m	4.51m	4.54m
12	3.87m	3.84m	3.86m	-	4.77m	4.81dd (9.8,5.9)
15	5.23d (2.9)	4.00d (2.9)	5.22d (2.9)	5.36d (2.7)	5.22d (2.7)	5.27d (2.7)
16	4.18dd (7.3,2.9)	4.14dd (7.3,2.9)	4.20dd (7.3,2.9)	4.14dd (6.6,2.7)	4.20dd (7.3,2.7)	4.21dd (7.1,2.7)
17	2.30d (7.3)	2.26d (7.3)	2.30d (7.3)	2.25d (6.6)	2.25d (7.3)	2.25d (7.1)
19	0.47d (4.6)	0.46d (4.6)	0.48d (4.3)	0.51d (4.4)	0.53d (4.6)	0.53d (4.6)
	0.60d (4.6)	0.58d (4.6)	0.62d (4.3)	N.O.	0.62d (4.6)	0.61d (4.6)
OAc	2.06s		2.05s	2.06s	2.06s	2.05s
			2.05s	2.06s	2.06s	2.06s
					2.06s	2.06s
26						1.81s
27						4.89m
						5.22d (1.7)

N.O.: Not observed



A



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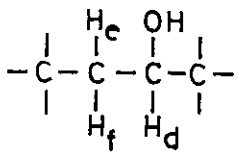

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structure A was considered to be located in the ring D of 9, 19-cyclolanostane structure. These findings indicate that **2** has a partial structure B. From the two-dimensional ^{1}H-^{13}C-correlation spectrum (^{1}H-^{13}C COSY) of 2 (Table III), ^{13}C-signals at δ 79.8, 86.7 and 44.1 were correlated with the ^{1}H-signals due to H_a (H-16), H_b (H-15) and H_c (H-17), respectively.

On treatment with p-toluenesulfonic acid, **2** gave a deacetyl compound (**3**), $C_{30}H_{48}O_6$, mp 163-166°C. In the ^{1}H-nmr spectra, the signal due to H-15 showed an upfield shift from δ 5.23 in **2** to 4.00 in **3**. The ^{1}H- and ^{13}C-nmr spectra of **3** indicated that no skeletal rearrangement had occurred during the acid treatment.

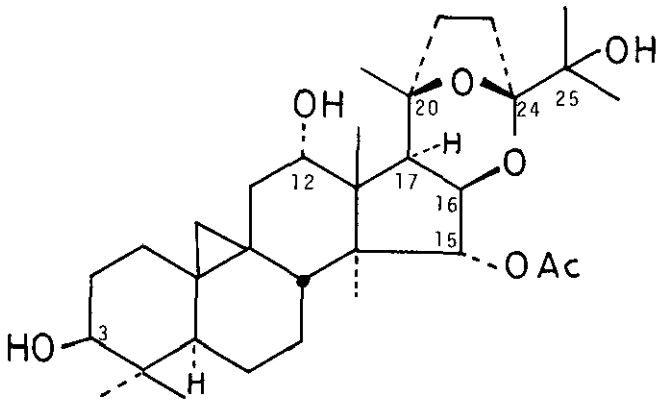
Acetylation of **2** afforded a monoacetate (**4**), $C_{34}H_{52}O_8$, mp 150-153°C. In the ^{1}H-nmr spectra, the signal due to H-3 showed a downfield shift from δ 3.28 in **2** to δ 4.54 in **4**. Compound **4** is 3-monoacetate of **2**. The ir spectrum of **4** still showed the presence of hydroxyl (3463 cm^{-1}). Jones oxidation of **4** afforded a ketone (**5**), $C_{34}H_{50}O_8$. In the ^{1}H-nmr spectrum of **4**, a multiplet due to methine attached to a hydroxyl group at δ 3.86 (in $CDCl_3$) was observed, but the spectrum of **5** lacked the signal. The ^{1}H-^{1}H-COSY of **2** (in d_5-pyridine) showed that a multiplet (H_d) at δ 4.08, which corresponded to that at δ 3.87 in $CDCl_3$ (Table II), was correlated with methylene protons, H_e and H_f at δ1.88 and 2.39 (J_{de}, J_{df}, J_{ef} = 8.9, 5.5, 14.8 Hz). These findings indicated that **2** has a partial structure C. The secondary alcohol group of the partial structure C must be located at C-11 or C-12. The cd spectrum of the ketone (**5**) showed a strong negative Cotton effect, $[\theta]$ - 6.1 x 10^3 at 292 nm, suggesting that the carbonyl group is at C-12.[1] It follows that the secondary hydroxyl group in **2** is at C-12. The ^{1}H-^{13}C COSY of **2** showed that a doublet at δ 72.2 (Table III) was correlated with the signal due to H-12.

On acetylation with acetic anhydride in pyridine under reflux, **4** afforded a diacetate (**6**), $C_{36}H_{54}O_9$. In the ^{1}H-nmr spectra, the signal due to H-12 showed a downfield shift from δ 3.86 in **4** to δ4.77 in **6**. On dehydration with $POCl_3$ in pyridine, **6** gave a dehydrate (**7**), $C_{36}H_{52}O_8$, which no longer showed a hydroxyl absorption in its ir spectrum. The ^{1}H-nmr spectrum of **7** showed signals ascribable to an isopropenyl group at δ1.81, 4.89 and 5.22. In **2**, the hydroxy isopropyl group must be located at the side chain. This result suggested that a tertiary hydroxyl group exists at C-25 in **2**.

Based on the unsaturation number (eight) in the molecular formula it was concluded that **2** has two additional rings to an acetyl carbonyl and the 9, 19-cyclolanostane ring system in its structure. The ^{13}C-nmr spectrum of **2** showed three signals

Table III. ^{1}H-^{13}C Heteroannular Correlation Spectral Data of Aglycone (2) in d_5-Pyridine

No.	H/C	δ_H	δ_C	H/C	δ_H	δ_C	H/C	δ_H	δ_C
1		ca.1.28	32.74	11		1.88	39.97	22	ca.1.72	40.22
		ca.1.58				2.39			ca.2.13	
2		ca.1.82	31.13	12		4.08	72.19	23	ca.2.05	28.63
		ca.1.95		13		-	49.80		2.56	
3		3.47	77.87	14		-	50.79	24	-	110.35
4		-	41.00	15		5.68	86.72	25	-	72.13
5		ca.1.35	47.48	16		4.54	79.75	26	1.63	25.17
6		0.76	21.37	17		2.77	44.14	27	1.74	24.48
		ca.1.52		18		1.59	20.52	28	1.46	25.17
7		ca.1.15	26.32	19		0.40	30.18	29	1.42	14.68
		ca.1.48				0.54		30	1.06	14.63
8		ca.1.78	48.21	20		-	82.92	OAc	2.07	169.94
9		-	19.90	21		1.19	26.06			21.37
10		-	26.50							



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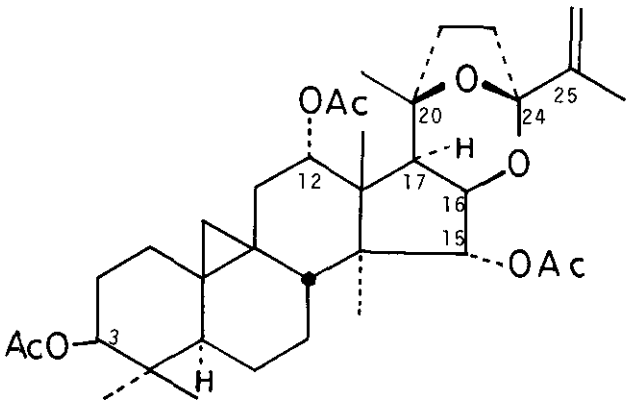

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attributable to oxygen-bearing quarternary carbons at δ 72.1, 82.9 and 110.4. The singlet at δ72.1 was assigned to C-25 in comparison with that of beesioside II. In the ^{1}H-nmr spectrum of **2**, any doublet methyl was not observed, suggesting that a proton usually present at C-20 of 9, 19-cyclolanostane terpenoid is substituted with an oxygen in **2**. The oxygen-bearing quaternary carbon at δ82.9 (C-20), the ketal carbon at δ110.4 (C-24) and the methine carbon at δ79.8 (C-16) suggested the presence of (20R, 24R or 20S, 24S)-16β, 24 and 20, 24 diepoxy structure in **2**. Consequently, the plane structure of the aglycone (**2**) was elucidated as **2**.

The determination of the stereochemistry at C-15, 20 and 24 was performed in comparison of the observed ^{1}H-^{1}H coupling constants ($J_{15,16}$=2.9, $J_{16,17}$=7.3 Hz) with those calculated by of Karplus equation[3] (Table IV). If C-20 and 24 have R configurations, the coupling constant between H-16 and H-17 should be 4.3 Hz (ring D in boat form) or 7.2 Hz (chair). If C-20 and 24 have S configulations, it should be 7.5 Hz (boat) or 7.0 Hz (chair). In the case of 20R and 24R, if H-15 is in α-side, the coupling constant between H-15 and H-16 should be 7.2 Hz (boat) or 4.7 Hz (chair), and if H-15 is in β, it should be 0.4 Hz (boat) or 7.2 Hz (chair). In the case of 20S and 24S, if H-15 is in α, it should be 5.4 Hz (boat) or 8.2 Hz (chair), and if H-15 is in β, it should be 7.5 Hz (boat) or 1.7 Hz (chair). Accordingly these configulations were determined as 20S, 24S and 15α-OAc.

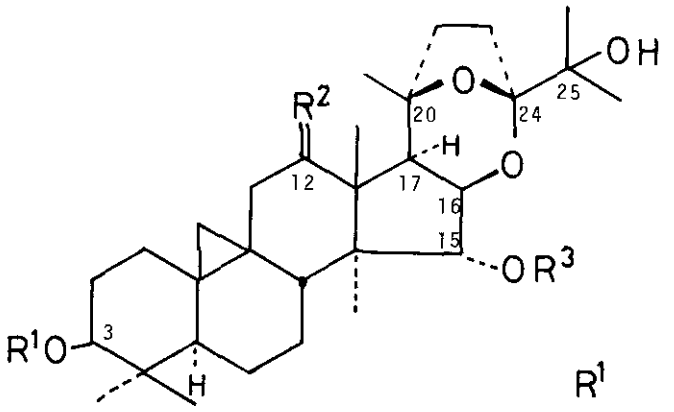
The configuration of C-12 was speculated on the comparison with that of a diacetate (**8**) of the aglycone of beesioside II, whose structure was elucidated by an X-ray structure analysis.[2] The torsion angle of C(9)-C(11)-C(12)-C(13) in **8** was - 14.4°. If 12-OH is in α-side, the coupling constants between H-12 and H_2-11 should be 7.7 and 4.4 Hz. If 12-OH is in β, they should be 7.7 and 0.4 Hz. In the ^{1}H-nmr spectrum of **2** (in d_5-pyridine), they were 8.9 and 5.5 Hz. It was conculuded that 12-hydroxyl group in **2** is located as α-substituent.

The sugar moiety of the xyloside (**1**) was shown to be attached to C-3 of the aglycone (**2**) as β-D-xylopyranoside by comparison of the ^{13}C-nmr chemical shifts of the anomeric carbon and C-3 in beesioside II with those of **1**. Application of Klyne's rule[4] to **1** and **2** also supported the β-D-xylopyranose structure [molecular rotation difference between **1** and **2**, -83.2°:methyl β-D-xylopyranoside, M_D -108°; methyl α-D-xylopyraniside, M_D + 253°].[5] Since ^{1}H-nmr spectrum of **1** showed the anomeric proton as a doublet (J= 6.2 Hz) at δ4.77, the xylose in **1** is β-anomer.

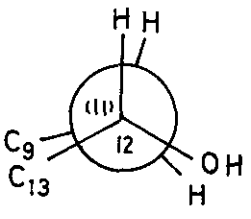
On the basis of the above described evidence and discussion we propose (20S, 24S)-15α-acetoxy-16β,24;20,24-diepoxy-9,19-cyclolanostane-3β,12α,25-triol 3-O-β-D-xylo-

Table IV. Calculated Dihedral Angles (Coupling Constants) between H-15, 16 and 17 in 2

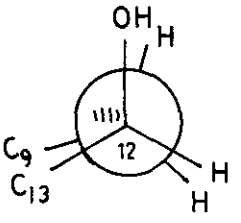
	20R, 24R		20S, 24S	
(J/Hz) \ ring D	boat	chair	boat	chair
H-17, H-16	43°(4.3)	20°(7.2)	15°(7.5)	22°(7.0)
H-16, H-15α	20°(7.2)	40°(4.7)	35°(5.4)	5°(8.2)
H-16, H-15β	105°(0.4)	160°(7.2)	155°(7.5)	117°(1.7)



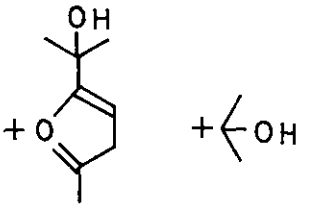
	R1	R2	R3
1:	β-D-xyl(p)	α-OH, H	Ac
2:	H	α-OH, H	Ac
3:	H	α-OH, H	H
4:	Ac	α-OH, H	Ac
5:	Ac	O	Ac
6:	Ac	α-OAc, H	Ac



12α - OH



12β - OH



ion a ion b

pyranoside for the structure of beesioside IV (1).

EXPERIMENTAL

Melting points were uncorrected. The following instruments were used: optical rotation; JASCO DIP-181 spectrometer: mass spectra; JEOL JMS-D 300 spectrometer: ir spectra; Hitachi 260-10 spectrophotometer: ^{1}H, and ^{13}C-nmr spectra; JEOL JNM-FX-100 and JEOL JNM-GX 400 spectrometers. Abbreviations: s=singlet, d=doublet, m=multiplet, br=broad.

Isolation of Beesioside IV (1)

In a previous paper[1], we described the isolation of 1 (50 mg) from the dried rhizomes of B. calthaefolia (39 g) and 1 (100 mg) from those of S. vaginata (90 g). **Beesioside IV (1)**: an amorphous powder. $[\alpha]_D^{20}$ - 11.1°(c=1.0, MeOH). Anal. Calcd for $C_{37}H_{58}O_{11}\cdot 3/2H_2O$:C, 62.97; H,8.71. Found:C, 63.33; H, 8.54. Irν_{max}^{KBr} cm^{-1}: 3400, 1720, 1245, 1040.^{1}H-Nmr (C_5D_5N) : 2.08 (3H,s, $OCOCH_3$), 4.77 (1H, d, J=6.2 Hz, anomeric H). ^{13}C-Nmr:Table I.

Enzymatic Hydrolysis of Beesioside IV (1)

Compound 1 (98 mg) in EtOH (49 ml) was treated with molsin (from Aspergillus saitoi) (98 mg) in a mixture of H_2O (49 ml) and 0.2M Na_2HPO_4-0.1M citric acid buffer (pH 4.0) (98 ml), and the total mixture was incubated at 38°C for 24 h. After usual work-up, the crude product (76 mg) was purified by column chromatography (silica gel, benzene-EtOAc (1:1)), to give the aglycone (2, 75 mg), an amorphous powder, $[\alpha]_D^{22}$ + 1.6°(c=1.0, $CHCl_3$). Irν_{max}^{KBr} cm^{-1}: 3410, 1730, 1245, 1030. Ms m/z (%): 546 (M^+, 15), 528(20), 486(15), 141 (ion a, 20), 123(ion a - H_2O, 100), 59(ion b, 85). Hrms m/z: 546.3528 (Calcd for $C_{32}H_{50}O_7$, 546.3554), 123.0828(Calcd for $C_8H_{11}O$, 123.0811). ^{1}H-Nmr: Table I. ^{1}H-^{13}C-COSY: Table III.

Xylose was identified in the aq. layer by tlc and glc(as xylitol acetate).

Treatment of 2 with p-Toluenesulfonic Acid

An aglycone 2(6 mg) in MeOH (1 ml) was refluxed with p-TsOH (1 mg) for 11 h. Usual work-up afforded 3 (3 mg), colorless needles, mp 163-166°C($CHCl_3$). Ir$\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 1120. Ms m/z (%): 504 (M^+,12), 486(30), 364 (15), 346(15), 328(29), 59 (ion b, 70). Hrms m/z: 504.3428 (Calcd for $C_{30}H_{48}O_6$, 504.3449). ^{1}H-Nmr:Table II.

Acetylation of 2

A solution of 2 (42 mg) in pyridine (0.5 ml) was left standing at room temperature overnight. Usual work-up afforded 4 (38 mg), colorless needles, mp 150-153°C

(benzene-EtOAc (1:1)), Ir ν_{max}^{KBr} cm^{-1}: 3463, 1728, 1233, 1033. MS m/z (%): 588 (M^+, 0.2), 510 (20), 123 (70). Hrms m/z: 588.3670 (Calcd for $C_{34}H_{52}O_8$, 588.3663). ^{1}H-Nmr: Table II. ^{13}C-Nmr: Table I.

Jones oxidation of 4

A solution of **4** (13 mg) in acetone (1 ml) was oxidized with Jones reagent (0.5 ml) in a refrigerator for 1 h, to furnish **5** (2 mg), an amorphous powder. Ms m/z (%): 586(M^+, 0.3), 526 (15), 123 (80). Hrms m/z: 586.3504 (Calcd for $C_{34}H_{50}O_8$, 586.3504). ^{1}H-Nmr: Table II. Cd (c=9.9 x 10^{-4}, MeOH) $[\theta]_{292}^{25}$: -6.1 x 10^3 (negative maximum).

Acetylation of 4

A solution of **4** (30 mg) in pyridine (2 ml) was refluxed with Ac_2O (0.1 ml) for 2 h to furnish **6** (21 mg). Ms m/z (%): 630 (M^+,0.1), 570 (15), 510 (45). Hrms m/z: 630.3781 (Calcd for $C_{36}H_{54}O_9$, 630.3768). ^{1}H-Nmr: Table II.

Dehydration of 6 with $POCl_3$

Compound **6** (20 mg) was dissolved in pyridine (0.5 ml) and 10 drops of $POCl_3$were added to the solution. The mixture was left standing in a refrigerator overnight. Usual work-up afforded **7** (10 mg), an amorphous powder. Ms m/z (%): 612 (M^+, 0.15), 552 (20), 492 (25). Hrms m/z: 612.3663 (Calcd for $C_{36}H_{52}O_8$, 612.3662). ^{1}H-Nmr: Table II. Ir $\nu_{max}^{CCl_4}$ cm^{-1}: 1738, 1655, 1230, 890, (no OH absorption).

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REFERENCES

1 T. Inoue, N. Sakurai, M. Nagai, and P. G. Xiao, Phytochemistry, 1985, **24**, 1329.

2 N. Sakurai, M. Nagai, H. Nagase, K. Kawai, T. Inoue, and P. G. Xiao, Chem. Pharm. Bull., 1986, **34**, 582.

3 M. Karplus, J. Chem. Phys., 1959, **30**, 11.

4 W. Klyne, Biochem. J., 1950, **47**, XLI.

5 E. L. Eliel, N. L. Allinger, and G. A. Morrison, "Conformational Analysis", Willy-Interscience, New York, 1965, p. 388.

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