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BEESIOSIDE III, A CYCLOLANOSTANOL XYLOSIDE FROM THE RHIZOMES OF *BEESIA CALTHAEFOLIA* AND *SOULIEA VAGINATA**

TAKAO INOUE, NOBUKO SAKURAI, MASAHIRO NAGAI† and XIAO PEIGEN‡

Faculty of Pharmaceutical Sciences; †Institute of Medicinal Chemistry, Hoshi University, Tokyo 142, Japan; ‡Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Dong Bei Wang, Beijing, China

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Key Word Index—*Beesia calthaefolia*; *Souliea vaginata*; Ranunculaceae; 9,19-cyclolanostanol xyloside; beesioside III.

Abstract—The chemical constituents of *Beesia calthaefolia* and *Souliea vaginata* were examined. From the rhizomes of *B. calthaefolia*, four new 9,19-cyclolanostanol xylosides, named beesiosides I–IV were isolated. Beesiosides III and IV were found also in the rhizomes of *S. vaginata*. On the basis of chemical and spectral evidence the structure of beesioside III was established as 15α-acetoxy-20ξ_1,24ξ_2-epoxy-9,19-cyclolanostane-3β,12β,16β,25-tetraol-3-*O*-β-D-xylopyranoside.

INTRODUCTION

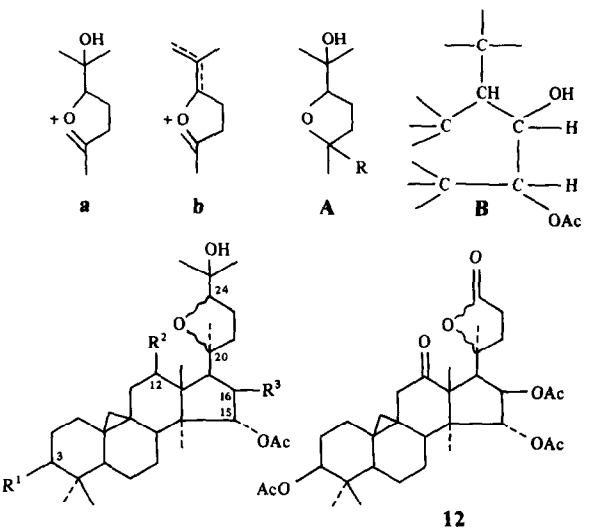
Beesia calthaefolia (Maxim.) Ulber. and *Souliea vaginata* (Maxim.) Franch. (both Ranunculaceae) have been used in China as herbal drugs for anti-inflammatory and analgesic effects. These plants are taxonomically close to the *Cimicifuga* genus and no phytochemical investigations have been made on them.

We have studied the constituents of a few species of *Cimicifuga* and isolated several 9,19-cyclolanostanol glycosides, including genuine glycosides easily convertible into some of other *Cimicifuga* glycosides, such as cimigenol xyloside [1]. It was of chemotaxonomical interest to study the constituents of *B. calthaefolia* and *S. vaginata*. This paper deals with the isolation of 9,19-cyclolanostanol glycosides, named beesiosides, from the above plants and the structure elucidation of beesioside III, a major glycoside of them.

RESULTS AND DISCUSSION

The ethyl acetate-soluble portion from a methanol extract of *B. calthaefolia* was successively subjected to silica gel and Sephadex LH-20 CC, affording beesioside I (**1**), beesioside II (**2**), beesioside III (**3**), beesioside IV (**4**) and sitosterol (**5**). Beesioside III (**3**), $C_{37}H_{60}O_{11} \cdot 0.5\,H_2O$, had mp of 178–182°, solidified at 220° and showed a mp 247–249°. The IR spectrum of **3** showed acetoxyl absorption bands (1710, 1270 cm^{-1}) together with strong hydroxyl bands due to its glycosidic structure [3450 (br), 1040 cm^{-1}]. Enzymatic hydrolysis of **3** with the crude glucosidase preparation, molsin, yielded an aglycone (**6**), $C_{32}H_{52}O_7$, and xylose. The high resolution mass spectrum of **6** gave two prominent fragments at *m/z* 143.1074, $C_8H_{15}O_2$, and *m/z* 125.0948, $C_8H_{13}O$, which were characteristic of the partial structure **A** in ocotillone-type triterpenes [2]. The ^1H NMR spectrum of **6** showed a double doublet at δ3.80 (*J* = 5.6 and 7.6 Hz) which was assignable to H-24 in **A**. Comparisons of the ^{13}C NMR data of **6** with those of the ocotillone triterpenes [3] also indicated the presence of the side chain **A** in **6**. ^1H and ^{13}C NMR examinations of **6** revealed that, along with the presence of the side chain **A**, an acetoxyl group and three secondary hydroxyl groups, **6** possessed seven tertiary methyl groups and one cyclopropane ring. The ^{13}C NMR spectrum of **6** showed signals identical with those of cycloartenol (**7**) [4] and cimigenol xyloside (**8**) [4], except for those signals due to C-12, the D-ring and the side chain, indicating that **6** should have a 9,19-cyclolanostane structure (Table 1).

In nuclear magnetic double resonance (NMDR) experiments with **6**, doublets at δ4.52 due to the proton on C-15



	R^1	R^2	R^3
3	O-β-xyl	OH	OH
6	OH	OH	OH
9	OAc	OH	OAc

*Part 1 in the series "Constituents of *Beesia calthaefolia* and *Souliea vaginata*".

Table 1. ^{13}C NMR chemical shifts of cycloartenol (7) [4], cimigenol xyloside (8), beesioside III (3), aglycone (6) and triacetate (9) (25.15 MHz in deuteropyridine)

Carbon No.	7	8	3	6	9
1	32.1	32.3	32.3	32.6	31.8
2	30.5	29.9	29.9	31.1	27.6
3	78.9	88.3	88.4	77.8	80.3
4	40.6	41.6	41.2	40.9	39.6
5	47.2	47.4	48.3	47.3	47.0
6	21.2	20.9	21.1	20.3	20.9
7	28.1	26.3	26.1	26.4	26.1
8	48.0	48.4	48.1	48.1	47.9
9	20.1	19.9	19.6	19.6	19.7
10	26.2	26.5	25.9	26.1	25.8
11	26.1	26.3	35.3	35.3	34.5
12	33.0	33.9	73.0	73.0	72.7
13	45.4	41.7	48.3	48.4	48.6
14	48.9	47.1	51.7	51.8	52.1
15	35.7	80.0	91.3	91.3	86.5
16	26.6	111.7	77.9	77.9	79.0
17	52.4	59.4	48.9	48.8	48.8
18	19.4	19.5	20.2	21.3	19.7
19	29.9	30.7	29.4	29.5	29.1
20	36.0	23.9	85.8	85.8	84.6
21	18.0	19.5	28.8*	27.4*	29.4*
22	36.5	38.0	36.5	36.6	36.0
23	25.0	71.7	26.3	28.8	26.2
24	125.4	90.0	83.1	83.1	83.1
25	130.8	70.9	69.9	69.9	69.9
26	17.6	25.3	27.5	26.1	27.4
27	25.7	25.6	27.5	26.1	27.7
28	25.5	26.8	25.7*	26.1*	25.6*
29	14.0	15.3	14.1	14.1	13.9
30	18.3	11.6	15.3	14.7	15.4
OAc	—	—	171.8	171.8	170.1
OAc	—	—	—	—	170.4
OAc	—	—	—	—	170.6
OAc	—	—	21.3	21.3	21.2
OAc	—	—	—	—	21.2
OAc	—	—	—	—	21.3
1′	—	107.2	107.3	—	—
2′	—	75.2	75.3	—	—
3′	—	78.3	78.3	—	—
4′	—	70.8	71.0	—	—
5′	—	66.8	66.9	—	—

*Values in any vertical column may be reversed although those indicated here are preferred.

and at δ2.90 due to the proton on C-17 changed into singlets upon irradiation of a double doublet at δ4.07 ascribable to the proton on C-16. On irradiation of the protons on C-15 or C-17, the H-16 signal changed into a doublet. Accordingly, an acetoxyl group in **6** was shown to be present at C-15, one of three secondary hydroxyl groups was shown to be at C-16 and the side chain was at C-17. These findings indicate that **6** has a partial structure **B**.

Acetylation of **6** afforded a 3,15,16-triacetate (**9**), which had another hydroxyl group. Oxidation of **9** with chromic acid in pyridine afforded a triacetyl monoketone (**10**). In the CD curve, **10** showed a negative Cotton effect, $[\theta]_{288}$ −9430. In dammarane triterpenes having a 12-keto group, the CD curves show a negative Cotton effect [5]. In *Buxus* alkaloids possessing an 11-keto group and a 9,19-cyclolanostane structure, the CD curves show a positive maximum [6]. In the ^1H NMR spectrum of **10**, signals due to an active methylene were observed at δ2.72 and 1.97 as an AB system (J = 20 Hz), but the spectrum of deuteriated **10** (**11**) [7, 8], lacked the AB type signals. Since the coupling constants (J = 5.7 and 8.1 Hz) between H_2-11 and H-12 were similar to those (J = 4 and 9 Hz) in acetyl acteol [9], the 12β-hydroxy configuration in **6** was substantiated.

Jones oxidation of the triacetate (**9**) afforded an acetyltrisnorketolactone (**12**). Its IR spectrum showed the presence of a five-membered lactone (1770 cm^{-1}). This evidence strongly suggested that the aglycone (**6**) had a side chain **A** [10].

The coupling constants ($J_{15,16}$ = 3.2 Hz and $J_{16,17}$ = 8.3 Hz) of H-16 supported the *cis*-relationship of the OH-16 group and the side chain **A**. Consequently, 15α-acetoxyl and 16β-hydroxyl configurations in **6** were substantiated. The signal due to H-3α in **6** was assigned from its chemical shift and coupling patterns.

From the data mentioned above the aglycone (**6**) was established to be a 9,19-cyclolanostane triterpene possessing OH-3β, OH-12β, OAc-15α and OH-16β groups, and the side chain **A**.

The sugar moiety of the xyloside (**3**) was shown to be attached to C-3 of the aglycone (**6**) as β-D-xylopyranose by comparison of the ^{13}C NMR chemical shifts of the anomeric carbon and C-3 in cimigenol xyloside (**8**) (Table 1) [4]. Application of Klyne's rule [11] to **3** and **6** also supported the β-D-xylopyranoside structure. Molecular rotation difference at 589 nm of **3** and **6**: $[\phi]$ −53.6°. Methyl β-D-xylopyranoside: $[\phi]$ −108°, methyl α-D-xylopyranoside: $[\phi]$ +253° [12].

Finally, the ^{13}C NMR examinations of beesioside III (**3**), the aglycone (**6**) and **9** demonstrated that acetylation shifts [13] were observed in the C-2–C-4, C-15 and C-16 signals for **6** and **9**, and that hydroxylation shifts [14] were observed in the C-11–C-13 and C-17 signals.

Accordingly, the structure of beesioside III (**3**) was established as 15α-acetoxy-20ξ_1,24ξ_2-epoxy-9,19-cyclolanostane-3β,12β,16β,25-tetraol-3-*O*-β-D-xylopyranoside.

From the ethyl acetate extract of *S. vaginata*, beesioside III (**3**) and beesioside IV (**4**) were isolated, and identified with **3** and **4** from *B. calthaefolia* by their mmp, IR and ^{13}C NMR spectra. The quantitative determinations of **2**–**4** in the rhizomes of *B. calthaefolia*, and **3** and **4** in those of *S. vaginata* were performed by reversed-phase HPLC.

Recently, Kusano and Nozoe [15] studied the constituents of the rhizomes of *Actaea asiatica*, taxonomically close to the *Cimicifuga* genus and isolated several triterpenoids related to cimigenol (**13**). The rhizomes of *B. calthaefolia* and *S. vaginata* do not contain **13** or its relatives.

As regards the chemotaxonomy of *Cimicifuga*, *Actaea*, *Beesia* and *Souliea*, from the results so far obtained, although these plants all contain 9,19-cyclolanostanol glycosides (mostly the xylosides), *Beesia* and *Souliea* are considered to be different from *Cimicifuga* and *Actaea* to some extent.

EXPERIMENTAL

All mps were uncorr. ORDs were measured using a 1 dm cell. ^1H NMR spectra were taken at 100 MHz in $CDCl_3$ soln using

TMS as int. standard. ^{13}C NMR spectra were recorded at 25 MHz in C_5D_5N (TMS as int. standard). The MS were run on a double focusing mass spectrometer and were recorded with an accelerating voltage of 3.0–6.5 kV and an ionizing potential of 70 eV. TLC was carried out on silica gel, Rp-18 (reversed-phase) and cellulose.

Plant material. Beesia calthaefolia was collected from Yunnan Province, and *Souliea vaginata* from Shensi Province, in China. Voucher specimens of these plants are deposited in the herbarium at the Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing, China.

Isolation of glycosides from B. calthaefolia. The air-dried, ground rhizomes (39 g) were extracted with MeOH. After removal of the solvent the residue (10 g) was extracted with EtOAc. The EtOAc extract was repeatedly chromatographed on silica gel and then on Sephadex LH-20 to give **1** (66 mg), **2** (30 mg), **3** (90 mg), **4** (50 mg) and sitosterol (**5**) (3 mg).

Beesioside III (**3**). Colourless needles, $[\alpha]_D^{16}$ +9.2° (MeOH; *c*0.8). IR ν_{max}^{KBr} cm^{-1}: 3450 (br), 1710, 1270, 1040. (Found: C, 64.57; H, 8.64. $C_{37}H_{60}O_{11}\cdot 0.5\ H_2O$ requires: C, 64.42; H, 8.91%.)

Enzymatic hydrolysis of **3**. Compound **3** (72 mg) in EtOH (10 ml) was treated with molsin (*Aspergillus saitoi*) (200 mg) in H_2O (10 ml) and 0.2 M Na_2HPO_4–0.1 M citric acid buffer (pH 4.0) (20 ml) and the total mixture was incubated at 37° for 14 hr. Usual work-up afforded **6** (50 mg), white powder, $[\alpha]_D^{20}$ +21.7° (MeOH; *c* 1.3). MS *m/z*: 548.3684 $[M]^+$ (calc. for $C_{32}H_{52}O_7$, 548.3711), 143.1074 (base peak) (calc. for $C_8H_{15}O_2$, 143.1072, ion a), 125.0948 (calc. for $C_8H_{13}O$, 125.0965, ion b). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3640, 3540, 1720, 1240, 1020. ^1H NMR: δ0.46, 0.54 (1H each, *d*, *J* = 5 Hz, H_2-19), 0.80, 0.97, 1.16, 1.17 (3H each), 1.29 (6H), 1.38 (3H) (all *s*, Me × 7), 2.11 (3H, *s*, OCOMe), 2.90 (1H, *d*, *J* = 8.3 Hz, H-17), 3.30 (1H, *m*, H-3), 3.68 (1H, *dd*, *J* = 5.7 Hz, *J* = 8.1 Hz, H-12), 3.80 (1H, *dd*, *J* = 7.6, 5.6 Hz, H-24), 4.07 (1H, *dd*, *J* = 8.3, 3.2 Hz, H-16), 4.52 (1H, *d*, *J* = 3.2 Hz, H-15). D-Xylose was identified as the sole sugar in the aq. layer by PC (*n*-BuOH–HOAc–H_2O, 6:1:2, R_f 0.20).

Acetylation of **6**. Compound **6** (18 mg) was acetylated with Ac_2O–pyridine (1:2, 2 ml) to give **9** (16 mg), colourless needles, mp 272–273° (EtOH), $[\alpha]_D^{16}$ +52.9° (MeOH; *c* 0.5). (Found: C, 68.26; H, 9.07. $C_{36}H_{56}O_9$ requires: C, 68.32; H, 8.92%.) MS *m/z*: 632 $[M]^+$, 143, 125. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3620, 3520, 1740, 1240. ^1H NMR: δ2.03, 2.04, 2.05 (3H × 3, *s*, OCOMe).

Oxidation of **9**. Compound **9** (7 mg) was oxidized with CrO_3 (10 mg) in pyridine (1 ml) to furnish **10** (4 mg), colourless needles, mp 227–228° (MeOH). MS *m/z*: 630 $[M]^+$, 612.3644 $[M-18]^+$ (calc. for $C_{36}H_{52}O_8$, 612.3660). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3520, 1740, 1717, 1240. ^1H NMR: δ1.97, 2.72 (1H each, *d*, *J* = 20 Hz, H_2-11). CD: $[\theta]_{288}$ −9430 (MeOH; *c* 5.37×10^{-4}).

Deuteriation of **10**. Compound **10** (2 mg) was refluxed with NaOAc (2 mg) and DOAc (1 ml) for 1.5 hr. Usual work-up afforded **11** (1 mg), mp 227–228°, colourless needles (MeOH). MS *m/z*: 617 $[M-15]^+$, 614.3808 $[M-18]^+$ (calc. for $C_{36}H_{50}O_8D_2$, 614.3788).

Jones oxidation of **9**. Compound **9** (10 mg) in Me_2CO (1 ml) was oxidized with Jones reagent (0.3 ml) at room temp. for 14 hr, to furnish **12** (3 mg), colourless needles, mp 285–287° (MeOH). MS *m/z*: 586.3090 $[M]^+$ (calc. for $C_{33}H_{46}O_9$, 586.3140). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1770, 1742, 1717, 1240. ^1H NMR: δ0.83, 0.86, 0.89, 1.47, 1.74 (3H each, all *s*, Me × 5), 2.00, 2.05, 2.06 (3H × 3, *s*, OCOMe), 2.73 (1H, *d*, *J* = 20.0 Hz, H-11), 2.98 (1H, *d*, *J* = 9.0 Hz, H-17), 5.39 (1H, *d*, *J* = 3.9 Hz, H-15), 5.58 (1H, *dd*, *J* = 9.0, 3.0 Hz, H-16).

Isolation of glycosides from S. vaginata. The air-dried ground rhizomes (90 g) were extracted with MeOH. After removal of the solvent the residue (29 g) was extracted with EtOAc. The EtOAc extract (4.4 g) was chromatographed repeatedly on silica gel (1: C_6H_6–EtOAc; 2: $CHCl_3$–MeOH–H_2O, 300:35:1; 3: C_6H_6–EtOAc–MeOH–H_2O, 40:40:8:1) to give **3** (375 mg), **4** (100 mg) and **5** (5.5 mg). Compounds **3** and **4** were identified with authentic **3** and **4** obtained from *B. calthaefolia* by mmp, IR and TLC.

Quantitative analysis of **2–4** *in* B. calthaefolia *and* S. vaginata. The powdered rhizomes of *B. calthaefolia* and *S. vaginata* were extracted × 4 (2 hr each) with MeOH under reflux. The combined soln was concd and the residue was extracted × 4 with EtOAc (2 hr each) under reflux. The combined soln was concd and the residue was washed with Et_2O to afford crude glycosides. Each of the beesiosides and crude glycosides were dissolved in the carrier solvent to *ca* 6 mg/ml. All the samples or standard solns were filtered through a TM-2P membrane filter (Toyo Roshi Co., Tokyo; pore size, 0.45 μm) before injection; column, Zorbax ODS (0.25 μm, 4.6 mm × 25 cm); temp., room temp.; mobile phase, MeOH–H_2O (85:15); flow rate, 1.2 ml/min; monitored with a differential reflectometer; sensitivity, 16×10^{-5} RIFS. Compound **2**: $y = 0.4328x - 3.422$ ($r = 0.99$); **3**: $y = 0.2932x - 3.861$ ($r = 0.99$); **4**: $y = 1.480x - 9.378$ ($r = 0.99$). Compound **2**: 0.16%, **3**: 0.27%, **4**: 0.19% in rhizomes of *B. calthaefolia*. Compound **3**: 0.32%, **4**: 0.24% in rhizomes of *S. vaginata*.

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