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NOTE

Preparation of menisdaurigenin and related compounds

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Abstract
Menisdaurin (**1**), a cyano glucoside, was first isolated in 1978 from *Menispermum dauricum* (Menispermaceae) and named after the plant. It has been also isolated from several plant sources. The stereochemistry of the aglycone part was first reported as (*Z*,4*R*,6*S*)-enantiomer of (4,6-dihydroxy-2-cyclohexen-1-ylidene)acetonitrile based on the CD spectrum of menisdaurilide (**2**), the α,β-unsaturated γ-lactone obtained by an acid hydrolysis of menisdaurin. Later, the absolute stereochemistry was revised as (*Z*,4*S*,6*R*) by X-ray crystal analysis of **1** isolated from *Saniculiphyllum guangxiens*. The aglycone part of menisdaurin (**1**) has not been obtained from **1**, because an acid hydrolysis of **1** gave menisdaurilide (**2**), and enzymatic hydrolysis with emulsin did not give the aglycone. On the other hand, a compound named coculauril (**3**) was isolated from *Cocculus lauriforius*. This compound has the same planner structure corresponding to the aglycone of **1**, but the stereochemistry was reported to be (*E*,4*R*,6*S*). Here, we confirmed the absolute stereochemistry of **1** by Mosher's method to be (*Z*,4*S*,6*R*), and prepared the aglycone of **1**, i.e., menisdaurigenin (**4**) by an enzymatic hydrolysis of **1**. We also revealed that **4** is a different compound from **3** and unstable in water and MeOH.

Keywords *Sinomenium acutum* · Menisdaurin · Menisdaurigenin · Enzymatic hydrolysis · Color change

Introduction

Menisdaurin (**1**), a cyano glucoside, was first isolated in 1978 from *Menispermum dauricum* (Menispermaceae) and named after the plant [1]. It has been also isolated from several plant sources [2–13]. The stereochemistry of the aglycone part was first reported as (*Z*,4*R*,6*S*)-enantiomer of (4,6-dihydroxy-2-cyclohexen-1-ylidene)acetonitrile based on the CD spectrum of menisdaurilide (**2**), the α,β-unsaturated γ-lactone obtained by an acid hydrolysis of **1** [1] (Fig. 1). The absolute stereochemistry of **2** isolated from *Sinomenium acutum* (Menispermaceae) was determined by X-ray crystal analysis of the *p*-bromobenzoate derivative of **2** as (4*S*,6*R*) [4], which suggested that **1** has the same (*Z*,4*S*,6*R*) stereochemistry. The absolute stereochemistry of **1** ($[\alpha]_D$ − 214.5°) itself was revised to (*Z*,4*S*,6*R*) based on detailed analyses of the NMR spectra including NOE effects between protons of the aglycone and the glucose moieties of **1** isolated from *Purshia tridentata* (Rosaceae) [5]. The absolute stereochemistry was also determined as (*Z*,4*S*,6*R*) by X-ray crystal analysis of **1** isolated from *Saniculiphyllum guangxiense* [10]. Recently, Li et al. [11] isolated **1** ($[\alpha]_D$ − 78.2°) from *Bruguiera gymnorrhiza* and presented the structure as (*Z*,4*R*,6*S*) citing the reports [2, 3] before the revision of the stereochemistry [5, 10].

The aglycone part of **1** has not been obtained from **1**, because an acid hydrolysis of **1** gave **2**, and enzymatic hydrolysis with emulsin did not give the aglycone [1]. On the other hand, a compound named coculauril (**3**) (Fig. 1) was isolated from *Cocculus lauriforius* [14]. This compound has the same planner structure corresponding to the aglycone of **1**, but the stereochemistry was reported to be (*E*,4*R*,6*S*). Here, we confirmed the stereochemistry of **1** and prepared its aglycone, i.e., menisdaurigenin (**4**), and related compounds, and examined their properties.

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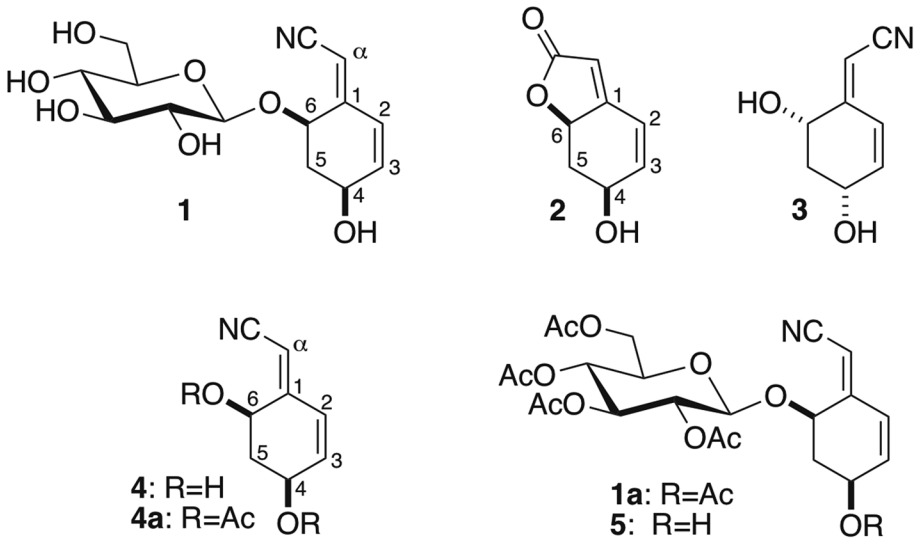

Fig. 1 Structures of menisdaurin (**1**) and related compounds

Results

We isolated menisdaurin (**1**) from a crude drug Boi (Sinomenium stem and rhizome: climing stem and rhizome of *Sinomenium acutum*). The isolated compound showed a negative sign of optical rotation ($[\alpha]_D$ − 223.2°, *c* 0.1, MeOH) as reported previously [1, 4, 5, 8] and its NMR spectral data were also in good agreement with those reported previously[1, 5]. Although the absolute stereochemistry of **1** isolated from *S. guangxiense* was determined by X-ray crystal analysis [10], the optical rotation of the material was not reported. Therefore, we first confirmed the absolute stereochemistry of **1** isolated from *S. acutum*. Menisdaurin (**1**) was acetylated to give its pentaacetate (**1a**), and the acetyl group on the allylic alcohol was site-selectively removed by enzymatic hydrolysis [15]. The lipase-catalyzed deacetylation was expected to proceed leaving acetyl groups attached to the glucose moiety intact [16]. Menisdaurin acetate (**1a**) was stirred with lipase B immobilized on acrylic resin from *Candida antarctica* in 2-propanol at 65 °C to give 4-*O*-deacetyl menisdaurin acetate (**5**). The position of the deacetylation was confirmed by the upper field shift of the H-4 proton (δ_H 4.18, br t, J = 8.4 Hz) compared to that of **1a** (δ_H 5.36, t, J = 3.9 Hz) (Table 1). In order to determine the absolute stereochemistry, we applied Mosher's method [17] to **5**. (*R*)- and (*S*)-MTPA esters of **5** were prepared according to the reported method and the ^{1}H-NMR spectrum of each ester was determined. The $\Delta\delta$ values ($\delta_S - \delta_R$) (Fig. 2) indicated that the absolute configuration at C-4 of **5** is (*S*), confirming the reported (4*S*,6*R*) absolute stereochemistry of **1** [5, 10].

An acid hydrolysis of **1** only gave **2**, and enzymatic hydrolysis of **1** with emulsin at pH 4.8 also failed to give the aglycone [1]. In fact, prolonged enzymatic hydrolysis of **1** gave a dark colored complex mixture, suggesting that the aglycone is unstable in an aqueous solution. To obtain the aglycone, **1** was hydrolyzed with β-glucosidase in sodium acetate buffer (pH 4.8) in the presence of ethyl acetate, by which the aglycone was continuously extracted. The aglycone, menisdaurigenin (**4**) was obtained under this condition in a good yield (87%). The structure of **4** was confirmed by its spectral data (Table 2). The *cis* relative sterochemistry between the two hydroxy groups and (*Z*) configuration of the double bond were confirmed by the nuclear Overhauser effect spectroscopy (NOESY) correlations between H-4 (δ_H 4.42, m) and H-6 (δ_H 4.70, br d, J = 7.3 Hz), and between H-2 (δ_H 6.26, d, J = 10.1 Hz) and H-α (δ_H 5.49, s), respectively (Fig. 3). Menisdaurigenin (**4**) has the opposite sign of optical rotation ($[\alpha]_D$ − 167.2°) to that of coculauril (**3**) ($[\alpha]_D$ + 94.0°) and its spectral data were different from those of **3** [14] (Table 2). These results indicated that **3** has the (*E*,4*R*,6*S*) stereochemistry as reported.

Menisdaurigenin (**4**) is unstable in water: the color of the solution changed to dark brown within 1 h and its spot had disappeared in TLC analysis. Compound **4** was less unstable in MeOH than in water. Figure 4 shows the color change of a solution of **4** in MeOH-d_4. The color of the solution changed to light brown after 22 h (Fig. 4) and small signals other than those of **4** appeared in the ^{1}H-NMR. After 39 h, the color became dark and the signals of **4** disappeared in the ^{1}H-NMR (Fig. 5b). The remaining signals (arrowed in Fig. 5b, Table 3) suggested that this compound might be a 4-oxo derivative of **4** (**6**) (Fig. 6). Dehydration of this compound will give a quinone methide (**9**), which undergoes polymerization to give the dark color. The involvement of the hydroxy groups of **4** in the

Table 1 NMR data of menisdaurin acetate (**1a**), 4-*O*-deacetyl menisdaurin acetate (**5**) and 4-oxomenisdaurin acetate (**7**) in $CDCl_3$

Position	**1a**		**5**		**7**	
	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
CN	–	116.5	–	116.5	–	115.3
α	5.36, s	99.4	5.30, s	98.4	5.65, s	100.8
1	–	152.8	–	153.5	–	151.5
2	6.35, d, *J* = 10.0 Hz	129.1	6.23, d, *J* = 10.0 Hz	125.6	6.21, d, *J* = 10.4 Hz	132.3
3	6.17, dd, *J* = 10.0, 4.5 Hz	132.9	6.34, dd, *J* = 10.0, 5.2 Hz	137.5	7.05, ddd, *J* = 10.0, 1.6, 0.8 Hz	140.3
4	5.36, t, *J* = 3.9 Hz	64.1	4.18, br t, *J* = 8.4 Hz	62.0	–	193.8
5 eq	2.23, dt, *J* = 14.2, 3.9 Hz	32.9	2.36, dt, *J* = 15.2, 2.0 Hz	35.0	2.86, dd, *J* = 16.4, 2.4 Hz	43.0
5ax	2.13, m		1.94, ddd, *J* = 15.2, 5.6, 2.8 Hz		2.72, dd, *J* = 16.8, 3.6 Hz	
6	4.76, br t, *J* = 4.0 Hz	73.6	4.88, br s	75.7	5.12, br dd, *J* = 5.2, 3.2 Hz	75.0
–OH			2.62, d, *J* = 11.6 Hz			
1′	4.86, d, *J* = 8.1 Hz	102.3	4.80, d, *J* = 8.0 Hz	102.5	4.75, d, *J* = 8.0 Hz	103.3
2′	5.06, dd, *J* = 9.5, 7.8 Hz	68.2	4.96, dd, *J* = 9.6, 8.0 Hz	71.1	4.90, dd, *J* = 9.6, 8.0 Hz	70.1
3′	5.20, t, *J* = 9.6 Hz	72.9	5.25, t, *J* = 9.6 Hz	72.2	5.20, t, *J* = 9.6 Hz	72.3
4′	5.09, t, *J* = 9.6 Hz	71.2	5.07, t, *J* = 10.0 Hz	68.0	5.07, t, *J* = 10.0 Hz	68.0
5′	3.77, ddd, *J* = 10.0, 4.4, 2.4 Hz	72.3	3.79, ddd, *J* = 10.4, 4.4, 2.4 Hz	72.3	3.75, ddd, *J* = 10.0, 4.4, 2.4 Hz	72.6
6′a	4.29, dd, *J* = 12.3, 2.4 Hz	61.7	4.33, dd, *J* = 12.0, 2.0 Hz	61.3	4.29, dd, *J* = 12.4, 2.4 Hz	61.4
6′b	4.12, dd, *J* = 11.9, 3.8 Hz		4.16, dd, *J* = 12.4, 4.0 Hz		4.18, dd, *J* = 12.4, 4.4 Hz	
$\underline{CH_3}CO$	2.09, s (6H)	20.6×2	2.10, s (6H)	20.6×2	2.10, s (3H)	20.6×2
	2.04, s (3H)	20.6	2.03, s (3H)	20.7	2.04, s (3H)	20.8
	2.02, s (3H)	20.8	2.01, s (3H)	20.8	2.02, s (3H)	
	2.00, s (3H)	21.0			1.99, s (3H)	
CO		169.0		169.4		169.2
		169.4		170.0		169.4
		170.3		170.1		170.2
		170.6		170.6		170.7
		171.0				

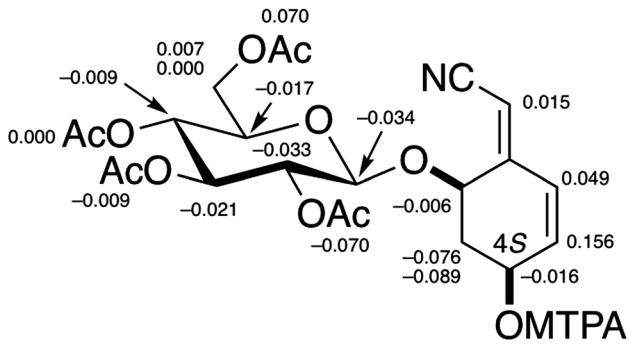

Fig. 2 $\Delta\delta$ values ($\delta_S - \delta_R$) of MTPA esters of **5**

color change was suggested by the fact that menisdaurigenin acetate (**4a**) was stable in MeOH.

As the intermediate could not be isolated, we oxidized the free alcohol of 4-*O*-deacetyl derivative **5** to examine its properties. Compound **5** was oxidized with pyridinium chlorochromate to give 4-oxomenisdaurin acetate (**7**). The structure was confirmed by the absence of the H-4 proton (δ_H 4.18, br t, *J* = 8.4 Hz) and C-4 carbon (δ_C 62.0) signals of **5** and the presence of a carbonyl carbon (δ_C 193.8, C-4) in **7** (Table 1).

Compound **7** was relatively stable in MeOH, but when **7** was heated under reflux in MeOH, it gave a new compound **8** after chromatographic separation. Compound **8** showed a pair of ortho coupled benzene protons (δ_H 6.82, d, *J* = 8.8 Hz, 7.30, d, *J* = 8.8 Hz, each 2H), a methoxy (δ_H 3.45, s, 3H) and a benzyl (δ_H 5.27, s, 1H) proton in the ^{1}H-NMR. These data are in good agreement with the reported data of compound **8** [18]. This compound may be formed as follows—elimination of the glucose moiety of **7** will give the quinone methide **9**, and an addition of MeOH to the δ position of α,β,γ,δ-unsaturated ketone of **9** will give the phenol **8** (Fig. 6). These results suggested that compound **9** is not involved in the dark color formation at least in MeOH and compound **6** and/or its isomer **6b** are taking place in the process.

In this report, we prepared menisdaurigenin (**4**) for the first time by enzymatic hydrolysis of menisdaurin (**1**). Menisdaurigenin was unstable in water and rapid

Table 2 NMR data of menisdaurigenin (**4**) and coculauril (**3**) in acetone-d_6

Position	**4**			**3**a)	
	^{1}H-NMR	^{13}C-NMR	HMBC	^{1}H-NMR	^{13}C-NMR
CN	–	117.6	g	–	117.5
α	g: 5.49, s	95.7	i, h	5.61, br s	91.7
1	–	159.8	a, b, g, i, h	–	161.6
2	i: 6.26, d, J = 10.1 Hz	127.0	g	6.53, dd, J = 10.1, 2.4 Hz	123.6
3	h: 6.22, dd, J = 10.1, 2.8 Hz	141.2	a, b	6.28, dq, J = 10.1, 1.7 Hz	143.9
4	d: 4.42, m	65.8	a, b, i, h	4.53, br m	67.3
5eq	b: 2.22, dt, J = 12.9, 4.3 Hz	40.7	i, h	2.43, dtd, J = 11.4, 4.9, 1.7 Hz	43.2
5ax	a: 1.94, ddd, J = 12.9, 9.1, 7.3 Hz			1.62, ddd, J = 12.8, 11.4, 10.1 Hz	
6	e: 4.70, br d, J = 7.3 Hz	67.6	a, b, g, i, h	4.41, br m	68.0
4-OH	c: 4.30, br s			4.45, d, J = 6.1 Hz	
6-OH	f: 4.93, br s			4.90, d, J = 6.1 Hz	

[a]Data from ref. [14]

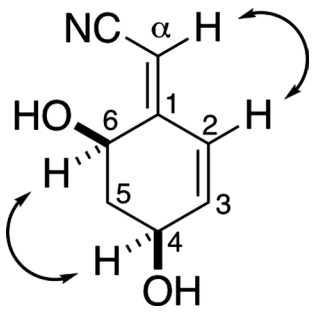

Fig. 3 Correlations observed in NOESY spectrum of menisdaurigenin (**4**)

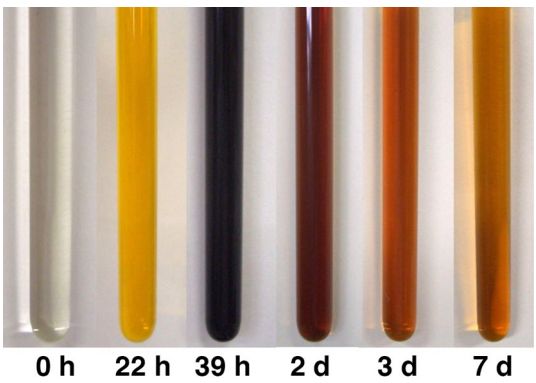

Fig. 4 Color change of MeOH solution of menisdaurigenin (**4**)

darkening of the solution was observed. The climbing stem and rhizome of *Sinomenium acutum* are used as a crude drug, Boi (Sinomenium stem and rhizome); there are two types of Boi—one has a relatively light color (white Boi) and the other has a dark color (black Boi). Comparison of the constituents of the two types showed that the content of alkaloids (sinomenine, magnoflorine and 6-*O*-methyllaudanosoline glucoside) together with menisdaurin (**1**) in black Boi was smaller than those in white Boi [19]. Black Boi is intentionally prepared by leaving collected stems and rhizomes in an open field for several months. It is reported that a rapid darkening of the cut surface was observed when the stems were cut after being left for a month [19]. Menisdaurigenin (**4**), which was formed by hydrolysis of **1** with an endogenous β-glucosidase during the period and oxidized on the cut surface, may take part in this darkening because, as described above, it is unstable and the color of its water solution darkened rapidly.

Experimental

Optical rotations were measured using a JASCO P-1020 (Tokyo, Japan). Infrared (IR) spectra were obtained with FT-IR SPX 60 (JASCO). TOF–MS was measured with a JMS-T100LP AccuTOF LC-plus (JEOL, Tokyo, Japan). EI-MS and HR-FAB-MS was measured with a JEOL JMS-7000. JEOL ECP-600 FT-NMR, VARIAN Unity Inova 500-MR (Palo Alto, CA, USA) and VARIAN 400-MR were used for measurement of NMR spectra with tetramethylsilane as an internal standard.

Column chromatography was performed with silica gel (40–50, 40–100, and 100–210 μm, Kanto Chemical, Tokyo, Japan), and Diaion HP-20 (Mitsubishi Chemical, Tokyo, Japan). Silica gel 60 F-254 was used for thin layer chromatography (TLC), and spots were detected under UV light (254 and/or 365 nm).

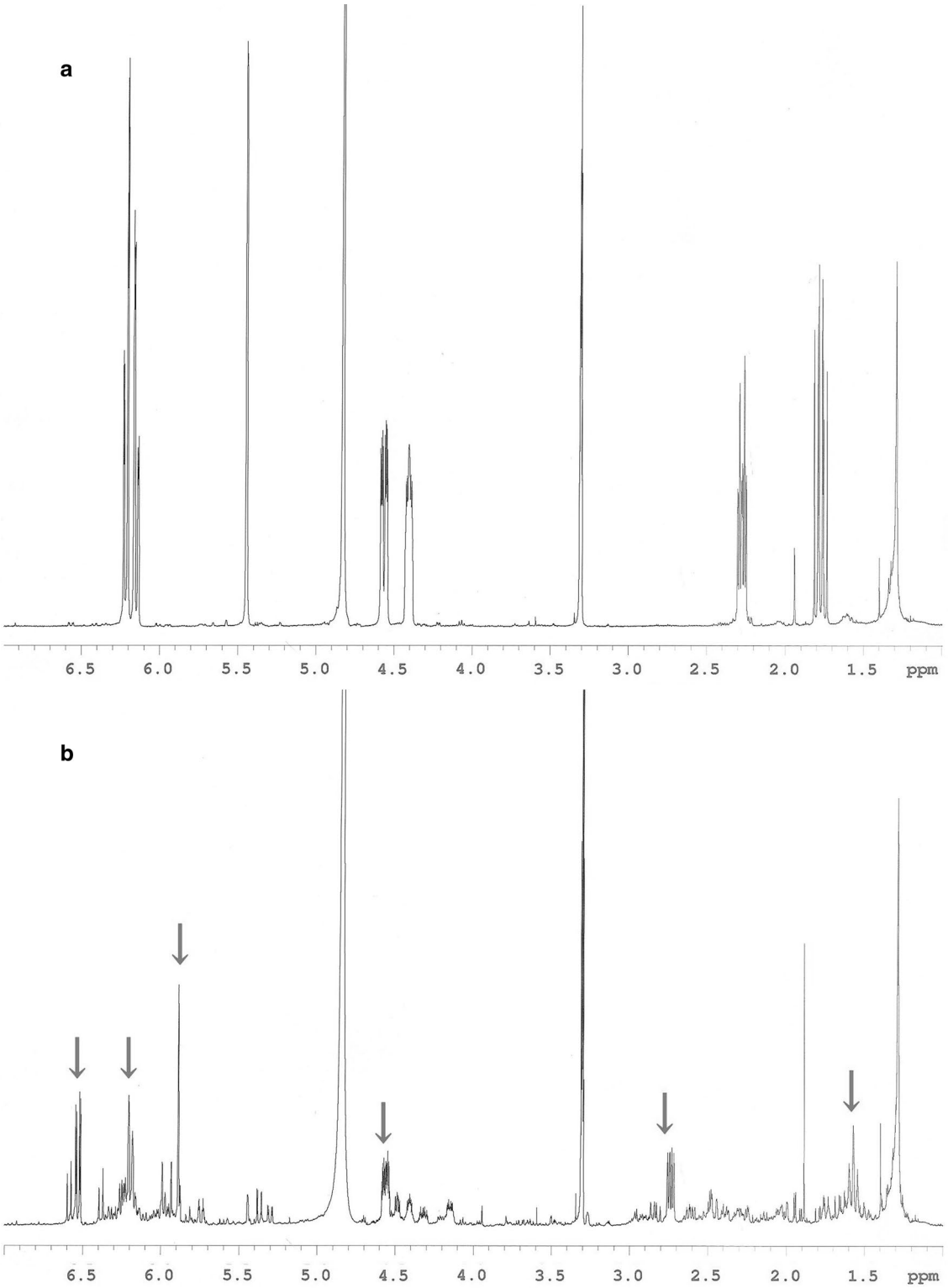

Fig. 5 Change of ^{1}H-NMR spectrum of menisdaurigenin (**4**) in MeOH. **a** Immediately after dissolved in MeOH; **b** 39 h after dissolved in MeOH. The arrows indicate the signals form the intermediate

Table 3 Comparison of ^{1}H-NMR data of the intermediate (**6**) with menisdaurigenin (**4**) and aglycone part of 4-oxomenisdaurin acetate (**7**) in MeOH-d_4

Position	4	6	7
α	5.44, br s	5.89, s	6.03, s
1	–	–	–
2	6.22, dd, J = 10.2, 1.4 Hz	6.19, dd like, J = 10.0, 1.6 Hz	6.15, dt, J = 10.0, 0.8 Hz
3	6.15, ddt, J = 10.0, 2.4 Hz, 1.2 Hz	6.53, dd, J = 10.0, 2.4 Hz	7.24, ddd, J = 10.0, 1.6, 0.8 Hz
4	4.40, m	–	–
5eq	2.27, br dd, J = 12.1, 4.6 Hz	2.74, dd, J = 10.8, 6.0	2.87, dd, J = 16.4, 3.2 Hz
5ax	1.77, ddd, J = 12.2, 11.4, 9.0 Hz	1.57, br t, J = 10.6 Hz	2.81, dd, J = 16.8, 3.2 Hz
6	4.56, ddd, J = 11.2, 4.4, 2.0 Hz	4.56, m	5.18, td, J = 3.2, 1.6 Hz

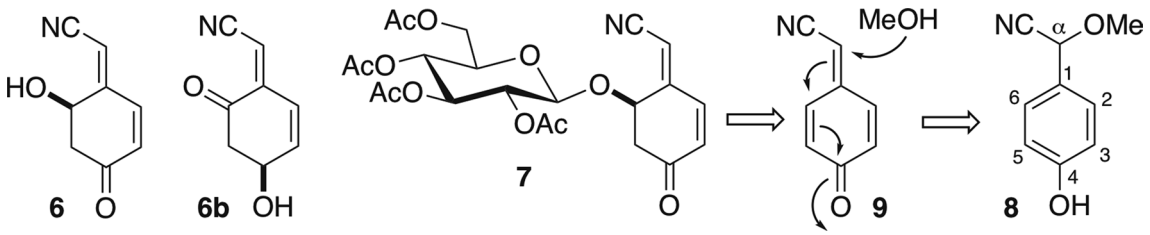

Fig. 6 Plausible structure of the intermediate (**6**) and change of 4-oxomenisdaurin acetate (**7**) in MeOH

Isolation of menisdaurin

Sinomenium stem and rhizome (100 g, Daikoshoyaku Co. Ltd., Lot 2G05) were extracted with MeOH (500 mL × 2) under reflux for 2 h and the extracts were concentrated under reduced pressure to dryness. The same procedure was repeated 6 times to give MeOH extract (20.55 g) from 600 g of the crude drug. The extract dissolved in water (200 mL) was extracted with chloroform (200 mL × 3). The water layer was concentrated to remove chloroform and applied to a column of Diaion HP-20 (5 cm × 20 cm), then successively eluted with water (1.2 L), 50% MeOH (1.6 L) and MeOH (0.9 L). The 50% MeOH eluate was concentrated to dryness and the residue (1.91 g) was chromatographed on silica gel (3 cm × 20 cm) with chloroform:MeOH = 4:1 (800 mL) to give menisdaurin (**1**) (344 mg).

Menisdaurin (1): white powder ($CHCl_3$-MeOH), mp 174 °C (lit. 174–175 °C), $[\alpha]_D^{22}$ − 223.2° (*c* 0.1, MeOH; lit. $[\alpha]_D^{25}$ − 212°, *c* 1.0, MeOH) [4]. MALDI-TOF–MS *m/z*: 336.7 $[M+Na]^+$. ^{1}H-NMR (600 MHz, MeOH-d_4) δ: 6.29 (1H, dd, J = 10.0, 1.0 Hz, H-2), 6.20 (1H, dd, J = 10.0, 3.6 Hz, H-3), 5.51 (1H, br s, H-α), 4.93 (1H, ddd, J = 8.4, 3.7, 1.5 Hz, H-6), 4.55 (1H, d, J = 7.4 Hz, H-1′), 4.36 (1H, br dd, J = 9.6, 4.9 Hz, H-4), 3.89 (dd, J = 12.0, 2.2 Hz, H-6 eq), 3.67 (1H, dd, J = 12.0, 6.3 Hz, H-6ax), 3.38 (1H, t, J=9.0 Hz,H-3′), 3.34 (2H, m, H-2′, 5′), 3.26 (1H, t, J = 9.0 Hz, H-4′), 2.27 (1H, ddd, J = 13.4, 4.9, 3.7 Hz, H-5 eq), 2.02 (1H, ddd, J = 13.4, 8.4, 6.3 Hz, H-5ax). ^{13}C-NMR (150 MHz, MeOH-d_4) δ: 157.1 (C-1), 140.6 (C-3), 127.7 (C-2), 118.0 (CN), 101.6 (C-1′), 96.8 (C-α), 78.2 (C-5′), 78.0 (C-3′), 74.5 (C-2′), 72.5 (C-6), 71.7 (C-4′), 65.4 (C-4), 63.2 (C-6′), 36.1 (C-5).

Acetylation of menisdaurin

A mixture of menisdaurin (**1**) (137 mg), acetic anhydride (1 mL) and pyridine (1 mL) was stirred at room temperature for 9.5 h. MeOH (1 mL) was added to the mixture under cooling with ice and the mixture was stirred at room temperature for 1 h. The mixture was concentrated to dryness and the residue was purified by silica gel column chromatography with hexane:ethyl acetate = 1:1 to give menisdaurin acetate (**1a**) (77.4 mg).

Menisdaurin acetate (1a): colorless needles (AcOEt-hexane), mp 176–178 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) and ^{13}C-NMR (100 MHz, $CDCl_3$): see Table 1.

Site-selective transesterification of menisdaurin acetate

Menisdaurin acetate (**1a**) (94.1 mg) in 2-propanol (18 mL) was stirred with lipase B immobilized on acrylic resin from *Candida antarctica* (185.3 mg) (Sigma-Aldrich, Lot SLBL2553 V) at 65 °C for 26.5 h. The mixture was filtered and the filtrate was concentrated to dryness. The residue was chromatographed on silica gel with hexane:ethyl

acetate = 1:2 to give 4-*O*-deacetyl menisdaurin acetate (**5**) (70.1 mg, 81.0%) with recovery of **1a** (5.5 mg).

4-*O*-deacetyl menisdaurin acetate (5): colorless needles (ethyl acetate-hexane), mp 136–138 °C. $[\alpha]_D^{22}$ − 105.4° (*c* = 0.1, MeOH). IR (ATP) cm^{-1}: 3500, 1741, 1361, 1224, 1043. MALDI-TOF–MS *m/z*: 504.45 $[M+Na]^+$. ^{1}H-NMR (400 MHz, $CDCl_3$) and ^{13}C-NMR (150 MHz, $CDCl_3$): see Table 1.

Preparation of (*S*)- and (*R*)-MTPA ester derivatives of 5

A mixture of **5** (2.0 mg, 4.2 µmol), DMAP (1.0 mg, 8.2 µmol), and (*S*)-MTPA-Cl (15 µL, 81 µmol) in pyridine (0.3 mL) was stirred for 15 h at room temperature. The reaction mixture was quenched by the addition of water and $CHCl_3$. The organic solvent was evaporated in vacuo. The residue was purified by silica gel column chromatography (hexane:ethyl acetate = 1:1) to give the (*R*)-MTPA ester derivative of **5** (2.1 mg, 71%) as colorless oil. ESI-TOF–MS *m/z*: 720.2 $[M+Na]^+$. ^{1}H-NMR (600 MHz, $CDCl_3$) *δ*: 7.53 (2H, br d, *J* = 7.9 Hz, phenyl of MTPA), 7.45–7.41 (3H, m, phenyl of MTPA), 6.28 (1H, d, *J* = 10.1 Hz, H-2), 6.04 (1H, d, *J* = 3.1 Hz, H-3), 5.64 (1H, br dd, *J* = 9.2,5.3 Hz, H-4), 5.34 (1H, s, H-α), 5.20 (1H, t, *J* = 9.4 Hz, H-3′), 5.13 (1H, t, *J* = 9.7 Hz, H-4′), 4.95 (1H, dd, *J* = 9.2, 8.4 Hz, H-2′), 4.87 (1H, d, *J* = 7.9 Hz, H-1′), 4.78 (1H, dd, *J* = 7.9, 4.0 Hz, H-6), 4.30 (1H, dd, *J* = 12.3, 2.2 Hz, H-6′b), 4.09 (1H, dd, *J* = 12.3, 4.4 Hz, H-6′a), 3.71 (1H, ddd, *J* = 9.4, 4.4, 2.6 Hz, H-5′), 3.53 (3H, s, OMe of MTPA), 2.43 (1H, dt, *J* = 13.6, 4.8 Hz, H-5 eq), 2.14 (1H, m, H-5ax), 2.09 (3H, s, 6′-OAc), 2.04 (3H, s, 4′-OAc), 2.00 (3H, s, 3′-OAc), 2.00 (3H, s, 2′-OAc).

Similarly, the (*S*)-MTPA ester derivative of **5** (2.3 mg, 75%) was prepared from **5** (2.1 mg) and (*R*)-MTPA-Cl. ESI-TOF–MS *m/z*: 720.2 $[M+Na]^+$. ^{1}H-NMR (600 MHz, $CDCl_3$) *δ*: 7.54 (2H, br d, *J* = 7.9 Hz, phenyl of MTPA), 7.44–7.40 (3H, m, phenyl of MTPA), 6.33 (1H, d, *J* = 10.1 Hz, H-2), 6.19 (1H, d, *J* = 3.5 Hz, H-3), 5.63 (1H, br dd, *J* = 9.0, 4.2 Hz, H-4), 5.35 (1H, s, H-α), 5.18 (1H, t, *J* = 9.2 Hz, H-3′), 5.12 (1H, t, *J* = 9.7 Hz, H-4′), 4.92 (1H, dd, *J* = 9.2, 8.3 Hz, H-2′), 4.84 (1H, d, *J* = 7.9 Hz, H-1′), 4.78 (1H, dd, *J* = 7.4, 4.0 Hz, H-6), 4.30 (1H, dd, *J* = 12.1, 2.4 Hz, H-6′b), 4.10 (1H, dd, *J* = 12.3, 4.4 Hz, H-6′a), 3.70 (1H, ddd, *J* = 10.1, 4.4, 2.6 Hz, H-5′), 3.54 (3H, s, OMe of MTPA), 2.34 (1H, dt, *J* = 13.7, 4.9 Hz, H-5 eq), 2.10 (3H, s, 6′-OAc), 2.07 (1H, m, H-5ax), 2.04 (3H, s, 4′-OAc), 1.99 (3H, s, 3′-OAc), 1.93 (3H, s, 2′-OAc).

Enzymatic hydrolysis of menisdaurin

A mixture of menisdaurin (**1**) (50 mg), β-glucosidase (20 mg, from almond, Tokyo Chemical Industries, Lot. 7V7YM, 3,000 U/mg), in 0.1 M acetate buffer (pH 4.8, 5.0 mL) and ethyl acetate (5 mL) was stirred at room temperature under argon for 1 h. The solution was extracted with ethyl acetate (5 mL × 4) to give menisdaurigenin (**4**) (21.0 mg, 87%).

Menisdaurigenin (4): white crystalline powder, mp 93–94 °C (decomp.). $[\alpha]_D^{26}$ − 167.2° (*c* 0.13, MeOH). IR (ATR) cm^{-1}: 3344, 3298, 3035, 2954, 2916, 2850, 2214, 1620, 1446, 1331, 1300, 1107, 1068, 1007, 856. MALDI–TOF–MS *m/z*: 152.5 $[M+H]^+$. ^{1}H-NMR (500 MHz, acetone-d_6) and ^{13}C-NMR (125 MHz, acetone-d_6): see Table 2. ^{1}H-NMR (400 MHz, MeOH-d_4): see Table 3.

Acetylation of menisdaurigenin

A mixture of menisdaurigenin (**4**) (15.9 mg), acetic anhydride (1.0 mL) and pyridine (1.0 mL) was stirred at room temperature for 7 h. The mixture was diluted with toluene and concentrated to dryness. The residue was purified by silica gel column chromatography (9 mm × 13 cm) with hexane:ethyl acetate = 3:1 to give menisdaurigenin acetate (**4a**) (13.6 mg, 86%).

Menisdaurigenin acetate (4a): White crystalline powder (ethyl acetate-hexane). mp 81–82 °C. IR (ATR) cm^{-1}: 2920, 2214, 1736, 1369, 1218, 1025. MALDI–TOF–MS *m/z*: 258.6 $[M+Na]^+$. ^{1}H-NMR (600 MHz, $CDCl_3$) *δ*: 6.27 (1H, dd, *J* = 10.0, 1.8 Hz, H-2), 6.14 (1H, dd, *J* = 9.9, 2.8 Hz, H-3), 5.82 (1H, ddd, *J* = 10.9, 4.5, 2.1 Hz, H-6), 5.55 (1H, ddd, *J* = 8.5, 5.8, 2.8 Hz, H-4), 5.32 (1H, s, H-α), 2.40 (1H, dt, *J* = 12.4, 5.0 Hz, H-5 eq), 2.21 and 2.08 (each 3H, s, -OAc), 2.02 (1H, ddd, *J* = 12.3, 10.9, 9.0 Hz, H-5ax). ^{13}C-NMR (150 MHz, $CDCl_3$) *δ*: 170.1 (–O<u>CO</u>Me × 2), 152.8 (C-1), 136.1 (C-3), 128.7 (C-2), 116.0 (CN), 96.5 (C-α), 67.2 (C-4), 66.8 (C-6), 33.8 (C-5), 21.0 and 20.7 (–OCO<u>Me</u>).

Oxidation of 4-*O*-deacetyl menisdaurin acetate (5)

A mixture of **5** (13.9 mg) and pyridinium chlorochromate 46.3 mg (TCI, P0930, Lot OMMRC-QE) in acetone (5 mL) was stirred at room temperature for 92 h. The mixture was concentrated to dryness and the residue was purified by silica gel column (17 mm × 10 cm) with hexane:ethyl acetate = 1:2 to give 4-oxomenisdaurin acetate (**7**) (12.7 mg, 91.8%).

4-Oxomenisdaurin acetate (7): pale yellow oil; ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$): see Table 1. ^{1}H-NMR (400 MHz, MeOH-d_4) δ: 7.24 (1H, ddd, *J* = 10.0, 1.6, 0.8 Hz, H-3), 6.15 (1H, dt, *J* = 10.0, 0.8 Hz, H-2), 6.03 (1H, s, H-α), 5.26 (1H, t, *J* = 9.4 Hz, H-3′), 5.18 (1H, td, *J* = 3.2, 1.6 Hz, H-6), 5.00 (1H, t, *J* = 10.0 Hz, H-4′), 4.92 (1H, d, *J* = 8.0 Hz, H-1′), 4.79 (1H, dd, *J* = 9.6, 8.0 Hz, H-2′), 4.25 (1H, dd, *J* = 12.4, 4.0 Hz, H-6b'), 4.19 (1H, dd, *J* = 12.4, 2.8 Hz, H-6a'), 2.87 (1H, dd, *J* = 16.4, 3.2 Hz,

H-5 eq), 2.81 (1H, dd, $J = 16.8$, 3.2 Hz, H-5ax), 2.06, 2.00 and 1.95 (each 3H, s, acetyl). ^{1}H-NMR (400 MHz, acetone-d_6) δ: 7.32 (1H, ddd, $J = 10.0$, 2.0, 0.8 Hz, H-3), 6.15 (1H, dt, $J = 10.0$, 0.8 Hz, H-2), 6.12 (1H, s, H-a), 5.28 (1H, t, $J = 9.6$ Hz, H-3′), 5.23 (1H, dt, $J = 3.2$, 2.0 Hz, H-6), 5.02 (1H, d, $J = 8.0$ Hz, H-1′), 5.01 (1H, t, $J = 10.0$ Hz, H-4′), 4.83 (1H, dt, $J = 9.6$, 8.0 Hz, H-2′), 4.23 (1H, dt, $J = 12.0$, 4.8 Hz, H-6′b), 4.13 (1H, dt, $J = 12.0$, 2.4 Hz, H-6′a), 3.97 (1H, ddd, $J = 10.0$, 4.8, 2.4 Hz, H-5′), 2.94 (1H, dt, $J = 16.4$, 3.6 Hz, H-5 eq), 2.88 (1H, dt, $J = 3.2$, 0.8 Hz, H-5ax), 2.02, 1.99, 1.98, 1.93 (each 3H, s, -OAc).

Change of 4-oxomenisdaurin acetate (7) in MeOH

Freshly prepared 4-oxomenisdaurin acetate **7** (18.9 mg) in anhydrous MeOH (5 mL) was refluxed under Ar atmosphere for 21.5 h. The mixture was concentrated to dryness and chromatographed on silica gel (9 mm × 9 cm) with chloroform:MeOH = 40:1. The major product was purified by HPLC with acetonitrile:water = 2:3 to give **8** (9.0 mg).

Compound 8: EI-MS *m/z*: 163 [M^+], 132 (M^+–OMe). ^{1}H-NMR (600 MHz, MeOH-d_4) δ: 7.30 (2H, d, $J = 8.8$ Hz, H-2,6), 6.82 (2H, d, $J = 8.8$ Hz, H-3,5), 5.27 (1H, s, H-α), 3.45 (3H, s, OMe). ^{13}C-NMR (150 MHz, MeOH-d_4) δ: 130.8 (C-2,6), 126.1 (C-1), 118.9 (CN), 116.7 (C-3,5), 72.9 (C-α), 57.2 (OMe).

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