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STRUCTURES OF POTENT ANTIULCEROGENIC COMPOUNDS FROM *CINNAMOMUM CASSIA*

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Abstract: Three potent antiulcerogenic compounds, cassioside : (4S)-2,4-dimethyl-3-(4'-hydroxy-3'-hydroxymethyl-1'-butenyl)-4-(β-D-glucopyranosyl)methyl-2-cyclohexen-1-one, cinnamoside : (3R)-4-[(2'R, 4'S)-2'-hydroxy-4'-(β-D-apiofuranosyl-(1 → 6)-β-D-glucopyranosyl)-2',6',6'-trimethylcyclohexylidene]-3-buten-2-one, and 3,4,5-trimethoxyphenol-β-D-apiofuranosyl-(1 →6)-β-D-glucopyranoside were isolated from a hot water extract of Cinnamomi Cortex (the dried stem bark of *Cinnamomum cassia* Blume) and their structures were determined.

Chinese cinnamon has been used in traditional Chinese medicine as a diaphoretic, an antipyretic, and an analgesic, and also widely used as an aromatic stomachic or a spice. In the course of pharmacological analysis of the traditional Chinese prescription "Goreisan", an aqueous extract from Cinnamomi Cortex (the dried stem bark of *Cinnamomum cassia* Blume; "Kannan Keihi" in Japanese), one of the constituents of "Goreisan", was found to have potent antiulcerogenic activities in rats.[1,2]

This paper describes the structure determination of three antiulcerogenic compounds, I, II and III, isolated from a hot water extract of Cinnamomi Cortex by the activity-directed fractionation process. Among them, I and II are new compounds.

The hot water extract of Cinnamomi Cortex (50 kg) was concentrated and the resulting precipitates were removed by decantation. The supernatant was chromatographed on Amberlite XAD-2, then eluted with water and 40 % aqueous methanol stepwise. The methanol-soluble portion of 40% aqueous methanol eluate was repeatedly rechromatographed by high-performance liquid chromatography (HPLC) on ODS or silica gel. Three kinds of glycosides, I (5.2 mg), II (19.3 mg) and III (40.0 mg), were isolated as antiulcerogenic compounds.

Compound I, named cassioside, was obtained as a colorless resinous syrup, $C_{20}H_{32}O_9$, SIMS (m/z) : 417 $[M+H]^+$, $[\alpha]_D$ -25.2° (c=0.5, methanol). The IR spectrum showed the presence of a hydroxyl group (3350 cm^{-1}) and a conjugated carbonyl group (1650, 1600 cm^{-1}) and the latter was further

supported by the absorption maximum at 265 nm (ε 3600) in the UV spectrum. The ^{1}H-NMR spectrum (Table 1) exhibited a tertiary methyl signal at δ 1.15 (s), an olefinic methyl signal at δ 1.78 (d, J=1.0 Hz), a pair of *trans* olefinic proton signals at δ 5.66 (dd, J=8.5, 16.3 Hz) and 6.29 (dt, J=1.0, 16.3 Hz), and an anomeric proton signal at δ 4.38 (d, J=7.8 Hz) suggesting the presence of β-glucopyranosyl linkage. On the other hand, the ^{13}C-NMR spectrum (Table 2) revealed the presence of two methyl (δ 15.7, 23.3), two methylene (δ 33.8, 36.0), a methine (δ 50.3), three hydroxymethyl (δ 64.7 x 2, 78.9), four olefinic (δ 131.5, 134.3, 139.2, 164.6), a quaternary (δ 42.4), and a carbonyl (δ 207.3) carbons, and six additional carbons belonging to a glucopyranosyl residue (δ 63.5, 72.4, 75.7, 78.4, 78.6, 105.5). The number of unsaturated degrees calculated for the molecular formula ($C_{20}H_{32}O_9$) indicated that the compound I contains a ring structure other than the glucopyranoside ring.

The enzymatic hydrolysis of cassioside (I) with β-D-glucosidase afforded an aglycone, named cassiol (IV), a colorless resinous syrup, $C_{14}H_{22}O_4$, EI-MS (m/z): 254 [M]$^+$, $[\alpha]_D$ +8.6° (c=0.25, methanol), UV : λ_{max} 268 nm (ε 9600), IR: ν_{max} 3400 (OH), 1650, 1600 (conjugated carbonyl) cm^{-1}. The ^{1}H-NMR spectrum (Table 1) showed two methyl signals at δ 1.12 (s) and 1.81 (d, J=1.0 Hz), and a pair of *trans* olefinic proton signals at δ 5.67 (dd, J=8.4, 16.3 Hz) and 6.28 (dd, J=1.0, 16.3 Hz).

The H/H COSY spectrum of cassioside (I) especially showed two long range spin-spin couplings between an olefinic methyl signal at δ 1.78 (2-CH_3) and an olefinic proton signal at δ 6.29 (1'-H), and between a methyl signal at δ 1.15 (4-CH_3) and a methylene signal at δ 2.26 (5-H), respectively.

These data suggested the following partial structures : -(C=O)-C(CH_3)=C-CH=CH-CH(CH_2O-)$_2$, -CH_2-CH_2-C-CH_3, -C-CH_2O-, and a glucopyranosyl residue. In the long range C/H COSY spectrum, long range couplings between the olefinic proton signal at δ 6.29 (1'-H) and both of the carbon signals at δ 50.3 (C-3') and 134.3 (C-2) suggested that the conjugated dienone structure should be a transoid form.

Furthermore, long range couplings were also observed between the methyl proton signal at δ 1.78 (2-CH_3) and three carbon signals at δ 134.3 (C-2), 164.6 (C-3) and 207.3 (C-1), and between the methyl proton signal at δ 1.15 (4-CH_3) and three carbon signals at δ 42.4 (C-4), 78.9 (C_4-hydroxymethyl carbon) and 164.6 (C-3), suggesting that the quaternary carbon (C-4) is connected to both the olefinic carbon (C-3) and the C_4-hydroxymethyl carbon. From these results, the planar structure of aglycone moiety was deduced to be IV, as shown in Fig. 1.

Among three hydroxymethyl carbon signals of IV, one at δ 70.4 (C_4-hydroxymethyl carbon) was shifted downfield by 8.5 ppm owing to glycosylation shift in the ^{13}C-NMR spectrum of I, but the others at δ 64.5 (C-4', C_3'-hydroxymethyl carbon) were shifted downfield by only 0.2 ppm. Therefore, the glucosylated position was decided to be at the hydroxymethyl carbon linked with the ring.

The absolute configuration of cassiol (IV) was determined by comparison of the CD spectrum of a derivative (V) (Fig. 2) with that of methyl tetrahydrotrisporate-C (VI)[3] (Fig. 2), whose absolute configuration had been determined by X-ray crystallographic analysis. Since the Cotton effect of IV due to the enone moiety was very weak, IV was converted into the trimethyl ester (V) by the selective

Table 1. ¹H-NMR spectral data of cassioside (I) and cassiol (IV) (δ_{ppm}, J=Hz)

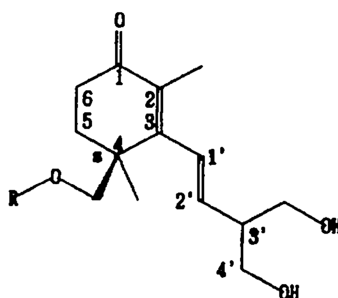
Proton	Cassioside (I) [a]	Cassiol (IV) [b]
2-CH_3	1.78 (d, 1.0)	1.81 (d, 1.0)
4-CH_3	1.15 (s)	1.12 (s)
4-CH_2O-	3.72 (d, 10.0)	3.43 (d, 11.5)
	3.80 (d, 10.0)	3.76 (d, 11.5)
5-H	1.77 (ddd, 5.5, 6.2, 13.2)	1.75 (ddd, 5.7, 6.4, 13.5)
5-H	2.26 (ddd, 5.5, 10.2, 13.2)	2.17 (ddd, 5.7, 10.0, 13.5)
6-H_2	2.55-2.68 (ddd, 5.5, 6.2, 10.2, 18.0)	2.51-2.69 (ddd, 5.7, 6.4, 10.0, 17.7)
1'-H	6.29 (dt, 1.0, 16.3)	6.28 (dt, 1.0, 16.3)
2'-H	5.66 (dd, 8.3, 16.3)	5.67 (dd, 8.3, 16.3)
3'-H	2.65 (td, 5.9, 7.0, 8.3)	2.67 (td, 6.0, 6.9, 8.3)
3'-CH_2O-	3.73 (dd, 5.9, 11.0)	3.75 (dd, 6.0, 11.1)
4'-H_2	3.65 (dd, 7.0, 11.0)	3.66 (dd, 6.9, 11.1)
glucose		
1-H	4.38 (d, 7.8)	
2-H	3.24 (dd, 7.8, 9.1)	
3-H	3.45 (dd, 9.0, 9.1)	
4-H	3.35 (dd, 9.0, 9.7)	
5-H	3.42 (td, 2.0, 6.0, 9.7)	
6-H	3.70 (dd, 6.0, 12.2)	
6-H	3.90 (dd, 2.0, 12.2)	

a) Measured in D_2O at 400 MHz., b) Measured in D_2O at 250 MHz.

Table 2. ¹³C-NMR spectral data of cassioside (I) and cassiol (IV) (δ_{ppm})

Carbon	Cassioside (I) [a]	Cassiol (IV) [b]
1	207.3	207.1
2	134.3	134.2
3	164.6	164.7
4	42.4	43.1
5	33.8	33.2
6	36.0	35.8
1'	131.5	131.3
2'	139.2	139.0
3'	50.3	50.1
4'	64.7	64.5
C_2- $\underline{CH_3}$	15.7	15.5
C_4- $\underline{CH_3}$	23.3	22.9
C_4- $\underline{CH_2}O$-	78.9	70.4
C_3' -$\underline{CH_2}O$-	64.7	64.5
glucose		
1	105.5	
2	75.7	
3	78.4	
4	72.4	
5	78.6	
6	63.5	

a) Measured in D_2O at 100.6 MHz.
b) Measured in $CDCl_3$-methanol-d_4 (1 : 1) at 50.3 MHz.



(I) Cassioside: R=β-D-Glu

(IV) Cassiol : R=H

Fig. 1.

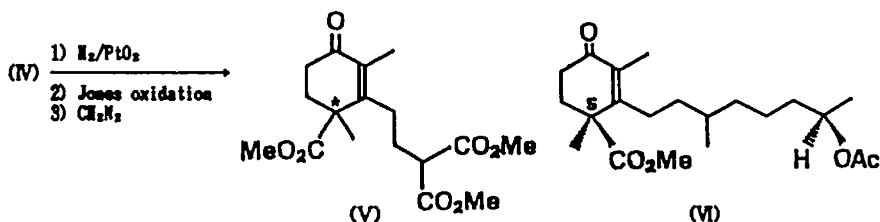


Fig. 2.

hydroganation, oxidation and methylation (overall yield : 26%). The CD spectrum of the β, γ-unsaturated ester (V) showed a negative Cotton effect, $[\theta]_{216}$ +15750, $[\theta]_{234}$ -21300, $[\theta]_{284}$ +640 and well coincided with that of VI, possessing S-configuration at C-4. Consequently, the structure of IV was determined to be (4S)-2,4-dimethyl-3-(4-hydroxy-3-hydroxymethyl-1-butenyl)-4-hydroxymethyl-2-cyclohexen-1-one. The complete structures of cassioside I [(4S)-2,4-dimethyl-3-(4-hydroxy-3-hydroxymethyl-1-butenyl)-4-(β-D-glucopyranosyl)methyl-2-cyclohexen-1-one)] was finally assigned as shown in Fig. 1.

Compound II was identified as 3,4,5-trimethoxyphenol-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside, which had been isolated from the aqueous extract of Cinnamomi Cortex (the dried stem bark of *Cinnamomum cassia* ; "Toko Keihi") by Miyamura *et al.*[4], by comparison of the ^{13}C-NMR spectral data with the reported data. The structure was shown in Fig. 3. However, its biological activity has not been reported up to date.

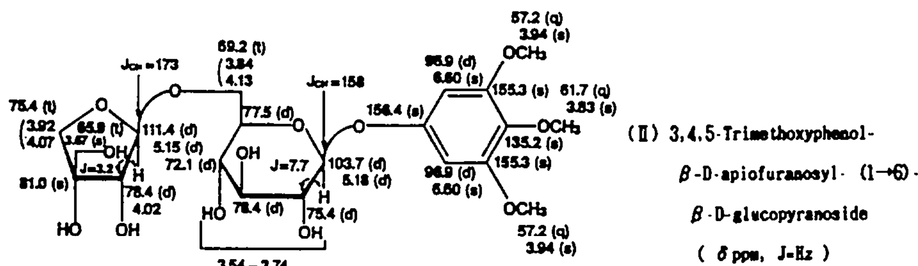


Fig. 3

Compound III, named cinnamoside, was obtained as a colorless resinous syrup, $C_{24}H_{38}O_{12}$, SIMS (m/z) : 519 [M+H]$^+$, $[\alpha]_D$ -88.6° (c=0.5, methanol). The UV spectrum showed an absorption maximum at 231 nm (ε 11700). The IR spectrum indicated the presence of a hydroxyl group (3350 cm^{-1}), an allenic moiety (1940 cm^{-1}) and a conjugated carbonyl group (1665, 1610 cm^{-1}). The ^1H-NMR spectrum (Table 3) exhibited three tertiary methyl signals at δ1.16, 1.33, 1.41 (each s), an acetyl methyl signal at δ2.26 (s), a methine signal on a hydroxy-bearing carbon atom at δ4.34 (dt, J=4.0, 11.6 Hz), two anomeric proton signals at δ4.60 (d, J=8.0 Hz) and δ5.09 (d, J=3.2 Hz), and an olefinic proton signal at δ5.95 (s) assignable to an allenic proton. The ^{13}C-NMR spectrum (Table 4) suggested that the sugar residue of cinnamoside (III) was identical with that of II, β-D-apiofuranosyl-β-D-glucopyranosyl group.

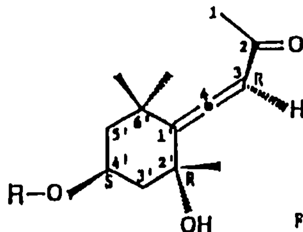


Fig. 4

Although cinnamoside (III) was resistant to acid hydrolysis, the enzymatic hydrolysis of III with the combined use of β-D-glucosidase and β-D-xylosidase afforded an aglycone (VII), $C_{13}H_{20}O_3$, MS (m/z) : 224 $[M]^+$, $[\alpha]_D$ -43° (c=0.23, methanol). The aglycone was considered to be grasshopper ketone, previously isolated from ant-repellant secretions of a large flightless grasshopper, *Romalea microptera*, by comparison of the ^{1}H-NMR spectral data (Table 3) with the reported data.[5] Based on the coupling constants (J=4.0, 11.5 Hz) of the methine proton at δ4.34 (dt), the configuration of the secondary hydroxyl group was determined to be equatorial. The configurations of the tertiary hydroxyl group and the allenic proton were considered to be the same with those of grasshopper ketone by comparison of chemical shifts in the ^{1}H-NMR spectral data with the reported data.[6,7,8] The absolute configuration of aglycone (VII) which showed a negative Cotton effect was determined to be identical with that of grasshopper ketone, whose absolute configuration had been determined by X-ray crystallographic analysis.[6,7] The aglycone (VII) was identified with the authentic grasshopper ketone, which was synthesized from isophorone according to the method of Weedon *et al.*[6] Signals of the carbon bearing a secondary hydroxyl group and the anomeric carbon of glucose were shifted downfield by 11.9 and 5.2 ppm, respectively, owing to glycosylation shift in the ^{13}C-NMR spectrum (Table 4) of III, whereas that of the carbon bearing a tertiary hydroxyl group was shifted downfield by only 2.0 ppm. Therefore, the anomeric carbon of the β-glucopyranosyl moiety is linked to the secondary hydroxyl group of the aglycone. The complete structure of cinnamoside (III) was determined to be (3R)-4-[(2'R, 4'S)-2'-hydroxy-4'-β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl-2',6',6'-trimethylcyclohexylidene]-3-buten-2-one, as shown in Fig.4.

Table 3. ^{1}H-NMR spectral data of cinnamoside (III), aglycone (VII) and grasshopper ketone (δ_{ppm}, J=Hz)

Proton	Cinnamoside (III) [a]	Aglycone (VII) [b]	Grasshopper ketone [c]
1-H_3	2.26 (s)	2.18 (s)	2.15 (s)
3-H	5.95 (s)	5.85 (s)	5.80 (s)
3'α-H	2.33 (ddd, 2.0, 4.0, 13.2)	2.30 (ddd, 2.0, 4.0, 13.0)	–
3'β-H	1.57 (dd, 11.5, 13.2)	1.43 (dd, 11.5, 13.0)	–
4'-H	4.34 (tt, 4.0, 11.5)	4.34 (tt, 4.0, 11.5)	4.28 (m, $w_{1/2}$~32Hz)
5'α-H	2.08 (ddd, 2.0, 4.0, 12.1)	2.00 (ddd, 2.0, 4.0, 13.0)	–
5'β-H	1.52 (dd, 11.5, 12.1)	1.36 (dd, 11.5, 13.0)	–
2'-CH_3	1.41 (s)	1.43 (s)	1.40 (s)
6'β-CH_3	1.16 (s)	1.16 (s)	1.16 (s)
6'α-CH_3	1.33 (s)	1.38 (s)	1.36 (s)
glucose			
1-H	4.60 (d, 8.0)		
2-H	3.24 (dd, 8.1, 9.2)		
3-H	3.49 (t, 9.2)		
4-H	3.40 (t, 9.4)		
5-H	3.60 (ddd, 2.0, 6.3, 9.6)		
6-H	3.72 (dd, 6.3, 11.4)		
6-H	4.03 (dd, 1.9, 11.4)		
apiose			
1-H	5.09 (d, 3.2)		
2-H	3.98 (d, 3.2)		
4-H	3.89 (d, 10.2)		
4-H	4.05 (d, 10.2)		
5-H_2	3.65 (s)		

a) Measured in D_2O at 400 MHz., b) Measured in $CDCl_3$ at 200 MHz., c) See references.

Table 4. ^{13}C-NMR spectral data of cinnamoside (III) and its aglycone (VII) (δ_{ppm})

Carbon	Cinnamoside (III) [a]		Aglycone (VII) [b]
1	33.4		31.8
2	214.2		209.7
3	102.9	(J_{CH}=171)	100.8
4	207.3		198.3
1'	120.7		118.8
2'	74.3		72.3
3'	47.2		48.8
4'	75.8		63.9
5'	48.6		49.0
6'	38.3		36.2
C_3'-CH_3	32.0		30.9
C_4'-CH_3	29.6		26.4
C_4'-CH_3	30.8		29.1
glucose			
1	103.7 [c]	(J_{CH}=161)	
2	75.7		
3	78.4		
4	72.4		
5	77.3		
6	70.3		
apiose			
1	111.6	(J_{CH}=172)	
2	78.4		
3	82.0		
4	76.3		
5	66.3		

a) Measured in D_2O at 100.6 MHz.
b) Measured in $CDCl_3$ at 50.3 MHz.
c) β-D-Glucose (C-1): 98.5ppm

EXPERIMENTAL

Infrared spectra (IR) were obtained with a Shimadzu Model IR-420 infrared spectrometer. Ultraviolet spectra (UV) were measured with a JASCO UVIDEC-505 digital spectrophotometer. Specific rotations were measured with a JASCO DIP-181 digital polarimeter. Circular dichroism spectra (CD) were recorded on JASCO J-20 spectrophotometer and JASCO J-500C spectropolarimeter. The EI-MS were recorded on a Shimadzu GC-MS QP-1000 spectrometer, and SIMS spectra were obtained with a Hitachi Model M80B spectrometer. Nuclear magnetic resonance spectra (NMR) were obtained with Bruker model AC-200, AM-250 or AM-400 spectrometer, and JEOL Model GX-270 spectrometer. Chemical shifts are reported in δ units with tetramethylsilane as an internal standard and coupling constants (J) are given in Hertz (Hz). Preparative and analytical high-performance liquid chromatographies (HPLC) were carried out with Waters System-500 and a chromatograph equipped with Waters Model M6000A pump, Waters Model U6K sample injector and JASCO UVIDEC-100-IV variable UV spectrophotometer. Columns used in the HPLC systems were YMC (Yamamura Chemical Laboratories Co., Ltd.)-ODS-A312 (150 mm x 6 mm), YMC-ODS-S343 (250 mm x 20 mm), Waters Prep-Pak C_{18} (300 mm x 50 mm) and Waters Vydac C_{18} (300 mm x 50 mm) for reverse phase separation, YMC-Silica-A012 (150mm x 6 mm), YMC-Silica-SH043 (250 mm x 20 mm), and Waters Prep-Pak Silica (300 mm x 50mm) for normal phase separation. Analytichem International's Bond Elut C_{18} (3 ml) was used for the reverse phase sample preparation. Thin layer chromatography (TLC) was performed on Merck precoated TLC plates of silica gel 60 F_{254}. Solvents were purified by standard procedures.

Extraction and Isolation Procedure :

Commercial Cinnamomi Cortex (the dried stem bark of *Cinnamomum cassia* Blume : " Kannan Keihi", 50 kg) was extracted twice with hot water (100 l each) at 100 ℃ for 1 hour. The combined extract (ED_{50}

58 mg/kg, *ip*, in serotonin-induced stomachic ulcer in rats) frozen at -22 ℃ was thawed slowly at room temperature, then centrifuged at 7000 rpm for 20 minutes to remove insoluble residues. The supernatant was admixed with 140 l of Amberlite XAD-2 ; the adsorbent was collected by filtration, washed with 300 l of water, and eluted with 300 l of 80% aqueous methanol as the eluent. The eluates concentrated to *ca* 100 l was subjected to Amberlite XAD-2 (*ca* 100 l) column chromatography, which was eluted with 40% aqueous methanol. The eluates were concentrated, and lyophilized to furnish 148 g of brown powder (ED_{50} 6.8 mg/kg, *ip*). This partially purified powder was dissolved in 200 ml of 10% aqueous acetonitrile, centrifuged at 10,000 rpm for 10 minutes to remove insoluble residues, and the supernatant was separated into three fractions by preparative HPLC on a Prep-Pak C_{18} column using 10% aqueous acetonitrile as the eluent. The fraction which exhibited an antiulcerogenic activity was collected, and concentrated under reduced pressure to give a brown resinous syrup (10 g). The syrup was suspended in 100 ml of methanol, then centrifuged at 10,000 rpm for 10 minutes. The methanol solution obtained by filtration was evaporated to give 8.5 g of pale brown resinous syrup (ED_{50} <0.5 mg/kg, *ip*), which was further purified by preparative HPLC on a Prep-Pak Silica column using chloroform-methanol-water (45 : 10 : 1) as the solvent system. The eluted fractions exhibiting antiulcerogenic activities were concentrated, and successively chromatographed on a Vydac C_{18} column using 8% aqueous acetonitrile as the eluent to give three active fractions (79.7 mg, 108.6 mg, and 356.7 mg), with ED_{50} values of less than 0.1 mg/kg, *ip*. These fractions were separately chromatographed on HPLC with a YMC-ODS-S343 column using 8% aqueous acetonitrile as the eluent and a YMC-Silica-SH043 column using chloroform-methanol-water (70 : 10 : 1) as the eluent, successively, to give compound I (5.2 mg, 0.00001%), compound II (19.3 mg, 0.00004%), and compound III (40.0 mg, 0.00008%), respectively.

Compound I :

Colorless resinous syrup ; $[\alpha]_D^{26.5}$ -25.2° (c=0.5, methanol) ; λ_{max}^{MeOH} nm (ε) : 265 (3600) ; ν_{max}^{film} cm^{-1}: 3350, 1650, 1600 ; SIMS (m/z) : 417 $[M+H]^+$ ($C_{20}H_{32}O_9$) ; ^1H-NMR (400 MHz, D_2O) : Table 1 ; ^{13}C-NMR (100.6 MHz, D_2O) : Table 2.

Enzymatic hydrolysis of compound I : Compound I (13.8 mg) was incubated with β-D-glucosidase (344 mg, 1720 units) in 12 ml of 50 mM citrate buffer(pH 4.6) at 37℃ for 91 hours. The reaction mixture was applied to a Bond Elut C_{18} (3 ml), and eluted with methanol. The methanol eluate was evaporated *in vacuo*, and the residue was dissolved in 50% aqueous methanol (1 ml), filtered, and subjected to HPLC on a YMC-ODS-S343 column using 8% aqueous acetonitrile as the eluent. The main peak fraction was collected, and concentrated *in vacuo* to give 5.9 mg of the aglycone (IV) as a colorless resinous syrup.

Aglycone of compound I : Colorless resinous syrup ; $[\alpha]_D^{28.5}$ +8.6 ° (c=0.25, methanol) ; λ_{max}^{MeOH} nm (ε) : 268 (9600) ; ν_{max}^{film} cm^{-1}: 3400, 1650, 1600, 1340, 1040, 980 ; MS (m/z) : 254 $[M^+]$, 236, 224, 206, 191, 179 ($C_{14}H_{22}O_4$) ; ^1H-NMR (250 MHz, D_2O) : Table 1 ; ^{13}C-NMR (50.3 MHz, $CDCl_3$-methanol-d_4=1 : 1) : Table 2.

Hydrogenation of compound IV : (4*S*)-2,4-dimethyl-3-(4-hydroxy-3-hydroxymethyl-butyl)-4-hydroxymethyl 2-cyclohexen-1-one (VII) : The suspension of compound IV (4 mg, 15.7 μmole) and 3 mg of platinum dioxide in 2 ml of ethanol under hydrogen atmosphere was stirred at room temperature for 40 minutes. After the catalyst was filtered off, the solvent was evaporated *in vacuo* and the residue was subjected to HPLC on a YMC-ODS-A312 column using 15% aqueous acetonitrile as the eluent. The main peak fraction was collected and the solvent was removed *in vacuo* to yield 2.9 mg (72 %) of colorless resinous syrup

; ν_{max}^{film} cm^{-1} : 3350, 1640, 1600, 1035 ; ^1H-NMR (200 MHz, methanol-d_4) δ_{ppm} : 1.15(3H, s), 1.51-1.79(4H, m), 1.79(3H, s), 2.13(1H, ddd, J=5.6, 7.6, 13.3 Hz), 2.33(1H, dd, J=4.3, 7.4 Hz), 2.38(1H, dd, J=4.3, 7.4 Hz), 2.40(1H, ddd, J=5.6, 7.6, 20.4 Hz), 2.54(1H, ddd, J=5.6, 9.1, 20.4 Hz), 3.46(1H, d, J=11.2 Hz), 3.61(4H, dt, J=1.2, 5.6 Hz), 3.66(1H, d, J=11.2 Hz) ; ^{13}C-NMR (50.3 MHz, methanol-d_4) δ_{ppm} : 11.9(q, C_2- $\underline{C}H_3$), 22.2(q, C_4- $\underline{C}H_3$), 28.0(t, C_1'), 29.6(t, C_2'), 33.2(t, C_5), 34.9(t, C_6), 42.9(s, C_4), 45.4(d, C_3'), 63.6(t, C_4'), 63.6(t, C_3'-$\underline{C}H_2O$-), 68.9(t, C_4'-$\underline{C}H_2O$-), 134.0(s, C_2), 165.7(s, C_3), 201.7 (s, C_1).

Jones oxidation and methylation of (VII): (4S)-2,4-dimethyl-3-(3,3-bis-methoxycarbonylpropyl)-4-methoxycarbonyl-2-cyclohexen-1-one (V) : To a stirred solution of 2.9 mg (11.3 μmole) of VII in 2 ml of acetone at 0°C was added dropwise 680 μmole of Jones reagent (CrO_3-H_2SO_4), and the mixture was stirred at 0°C for 30 minutes. Water (0.5 ml) was added, and the solution was extracted five times with chloroform. The combined chloroform layer was dried, filtered, and evaporated *in vacuo*, then the residue was dissolved in 1 ml of tetrahydrofuran. To the solution stirred at 0 °C was added an excess amount of ethereal diazomethane solution. After an additional 30 minute-stirring at 0°C, the reaction mixture was concentrated *in vacuo*. The residue was dissolved in 1 ml of 50% aqueous methanol, then filtered to remove insoluble residues. The filtrate was subjected to HPLC on a YMC-ODS-A312 column using 40% aqueous acetonitrile as the eluent. The main peak fraction was collected and the solvent was removed *in vacuo* to give 1.0 mg (26%) of colorless resinous syrup (V) : $[\alpha]_D^{25}$ -30° (c=0.02, methanol) ; λ_{max}^{MeOH} nm (ε) 243 (6160) ; ν_{max}^{film} cm^{-1} : 3000, 1730, 1665, 1615, 1250, 1200, 830 ; MS (m/z) : 340 $[M]^+$, 329, 281 ($C_{17}H_{24}O_7$) ; ^1H-NMR (200 MHz, $CDCl_3$) δ_{ppm} : 1.47(3H, s), 1.84(3H, s), 3.72(3H, s), 3.76(6H, s) ; CD (c=0.004, ethanol) : $[\theta]_{340}$ 0, $[\theta]_{320}$ +430, $[\theta]_{298}$ 0, $[\theta]_{284}$ +640, $[\theta]_{268}$ 0, $[\theta]_{236}$ -21300, $[\theta]_{222}$ 0, $[\theta]_{210}$ +15750.

Compound II :

Colorless resinous syrup ; $[\alpha]_D^{25}$ -99.8° (c=0.5, methanol) ; λ_{max}^{MeOH} nm (ε): 212 (9200), 270 (490) ; ν_{max}^{film} cm^{-1} : 3400, 1605, 1510, 825, 785 ; SIMS (m/z) : 479 $[M+H]^+$ ($C_{20}H_{30}O_{12}$) ; ^1H-NMR (270 MHz, D_2O) δ_{ppm} : 3.54-3.74(4H, m), 3.67(2H, s), 3.83(3H, s), 3.84(1H, d, J=9.2 Hz), 3.92(1H, d, J=10.6 Hz), 3.94(6H, s), 4.02(1H, d, J=3.0 Hz), 4.03(1H, d, J= 10.3 Hz), 4.13(1H, d, J=9.2 Hz), 5.15(1H, d, J=3.2 Hz), 5.18(1H, d, J=7.7 Hz), 6.60(2H, s) ; ^{13}C-NMR (67.9 MHz, methanol-d_4) δ_{ppm} : 57.2(q, C_3-$O\underline{C}H_3$), 57.2(q, C_5-O $\underline{C}H_3$), 61.7(q, C_4- $O\underline{C}H_3$), 65.9(t, api-C_5), 69.2(t, glu-C_6), 72.1(d, glu-C_4), 75.4(d, glu-C_2), 75.4(t, api-C_4), 77.5(d, glu-C_5), 78.4(d, glu-C_3), 78.4(d, api-C_2), 81.0(s, api-C_3), 96.9(d, C_2), 96.9(d, C_6), 103.7(d, glu-C_1), 111.4(d, api-C_1), 135.2(s, C_4), 155.3(s, C_3), 155.3(s, C_5), 156.4(s, C_1).

Compound III :

Colorless resinous syrup ; $[\alpha]_D^{24}$ -88.6° (c=0.5, methanol) ; λ_{max}^{MeOH} nm (ε): 231 (11700), 207 (9600) ; ν_{max}^{film} cm^{-1}: 3350, 1940, 1665, 1610, 864, 821 ; SIMS (m/z) : 519 $[M+H]^+$ ($C_{24}H_{38}O_{12}$) ; ^1H-NMR (400 MHz, D_2O) : Table 3 ; ^{13}C-NMR (100.6 MHz, D_2O) : Table 4.

Enzymatic hydrolysis of compound III : Compound III (10.2 mg) was incubated with β-D-glucosidase (100 units) and β-D-xylosidase (15 units) in 6 ml of 50 mM citrate buffer (pH 4.6) at 37°C for 44 hours. The reaction mixture was applied on a Bond-Elut C_{18} (3 ml). The methanol eluate was evaporated *in vacuo* ; the residue was dissoleved in 50% aqueous methanol (1 ml), and the filtrate was subjected to HPLC on a YMC-ODS-S343 column using 12% aqueous acetonitrile as the eluent. The main peak fraction collected was concentrated *in vacuo* to give 3.0 mg of the aglycone as colorless crystals.

Aglycone of compound III : Colorless crystals, mp 128 °C ; $[\alpha]_D^{29}$ -43° (c=0.23, methanol) ; λ_{max}^{MeOH}

nm (ε) : 230(12500) ; ν_{max}^{film} cm^{-1} : 3300, 1935, 1665, 1215, 1180, 1155 ; CD (c=0.158, dioxane) : $[\theta]_{300}$ 0, $[\theta]_{251}$ -10100, $[\theta]_{236}$ 0, $[\theta]_{225}$ +11900, $[\theta]_{212}$ 0, $[\theta]_{205}$ -8000 ; MS (m/z) : 224 $[M]^+$, 209, 191, 163, 123 ($C_{13}H_{20}O_3$) ; ^1H-NMR (200 MHz, $CDCl_3$) : Table 3 ; ^{13}C-NMR (50.3 MHz, $CDCl_3$) : Table 4.

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REFERENCES

1) S. Tanaka, T. Akira and M. Tabata, Yakugaku Zasshi, 104 , 601-606 (1984)

2) T. Akira, S. Tanaka and M. Tabata, Planta Medica, 440-443 (1986)

3) J. D. Bu'Lock, D. J. Austin, G. Snatzke and L. Hruben, J. Chem. Soc., Chem. Commun., 255-256, (1970)

4) M. Miyamura, T. Nohara, T. Tomimatsu and I. Nishioka, Phytochemistry, 22, 215-218 (1983)

5) J. Meinwald, K. Erickson, M. Hartshorn, Y. C. Meinwald and T. Eisner, Tetrahedron Lett., 2959-2962 (1968)

6) J. R. Hlubucek, J. Hora, S. W. Russell, T. P. Toube and B. C. L. Weedon, J. C. S. Perkin I, 848-852 (1974)

7) K. Mori, Tetrahedron, 30, 1065-1072 (1974)

8) S. Isoe, S. Katsumura, S. B. Hyeon and T. Sakan, Tetrahedron Lett., 1089-1092 (1971)