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NEW *NOR*-NEOLIGNAN GLYCOSIDES FROM *STYRAX OBASSIA* (STYRACACEAE)

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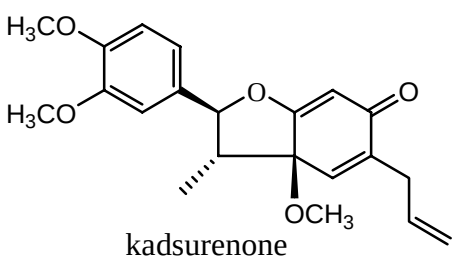
Abstract - Three new *nor*-neolignan glycosides, named obassiosides A, B and C, along with one known compound egonol-β-gentiobioside were isolated from the bark of *Styrax obassia* (Styracaceae), and their structures were deduced on the basis of the spectroscopic evidence.

Neolignan is a general name of secondary metabolites biogenetically resulting from condensation of two units of phenylpropanoid precursor (**1**; coniferyl alcohol) by oxidative coupling *via* radical-mediated mechanisms, and distinguished from lignan in that the linkage of phenylpropanoid units occurs also in the aromatic ring. Kadsurenone is perhaps the most well known compound among neolignans, which is isolated from *Piper futokadsura*[1] and otherwise known for its potent PAF antagonist activity. In terms of the drug development from natural product, kadsurenone and its analogues are considered to be promising seed chemicals. We have thus been inspired to search kadsurenone analogues, in particular, from natural source alternative to the genus *Piper* that is mostly of the tropical origin and is increasingly difficult to collect since the convention on biological diversity (CBD) was enacted in 1992. We selected as one of the alternative candidates the genus *Styrax* from which a number of *nor*-neolignans have been isolated.[2-7] The genus *Styrax* is chemically characterized by the occurrence of a 2-phenylbenzofuran derivative egonol (**4**).[2-7] Of particular interest to note is that the occurrence of dihydrodehydrodiconiferyl alcohol (**2**), a supposed biogenetic precursor of egonol (**4**) as illustrated in Fig. 1, was reported from *Styrax ferrugineus*.[5] This compound has a reduced benzofuran structure, the only example of this kind from the genus *Styrax* up to the present, which is more similar to kadsurenone structurally than other *nor*-neolignans commonly occurring in the genus *Styrax*. The genus *Styrax* consists of about one hundred species, among which only a few species have been chemically investigated. The following three species belonging to this genus are known to occur in Japan: *S. japonica* Sieb. et Zucc. (egonoki), *S. obassia* Sieb. et Zucc. (haku-unboku) and *S. shirainana* Makino (ko-haku- unboku).[8] Since only the fruit of *S. obassia* has been chemically



kadsurenone

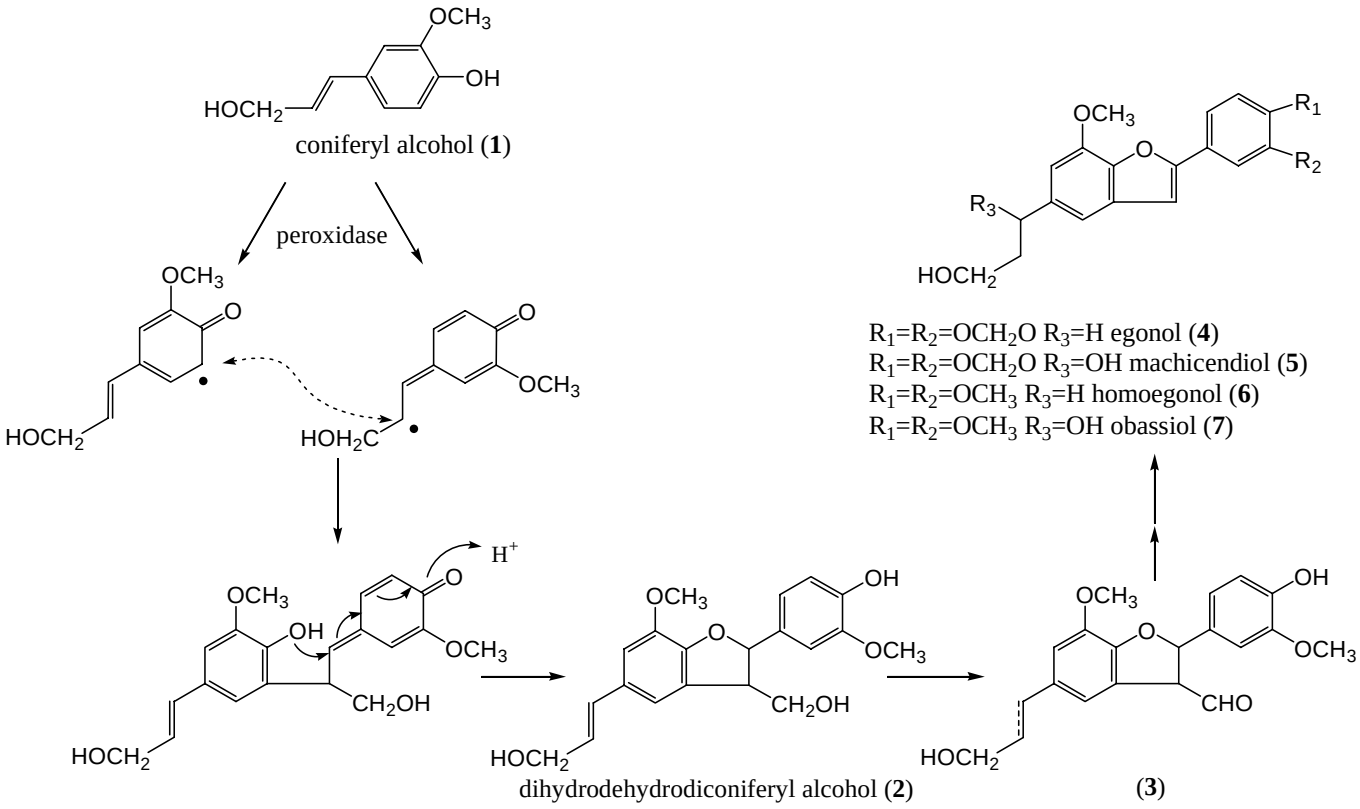


Figure 1 Possible biogenetic scheme of egonol (4) and related compounds

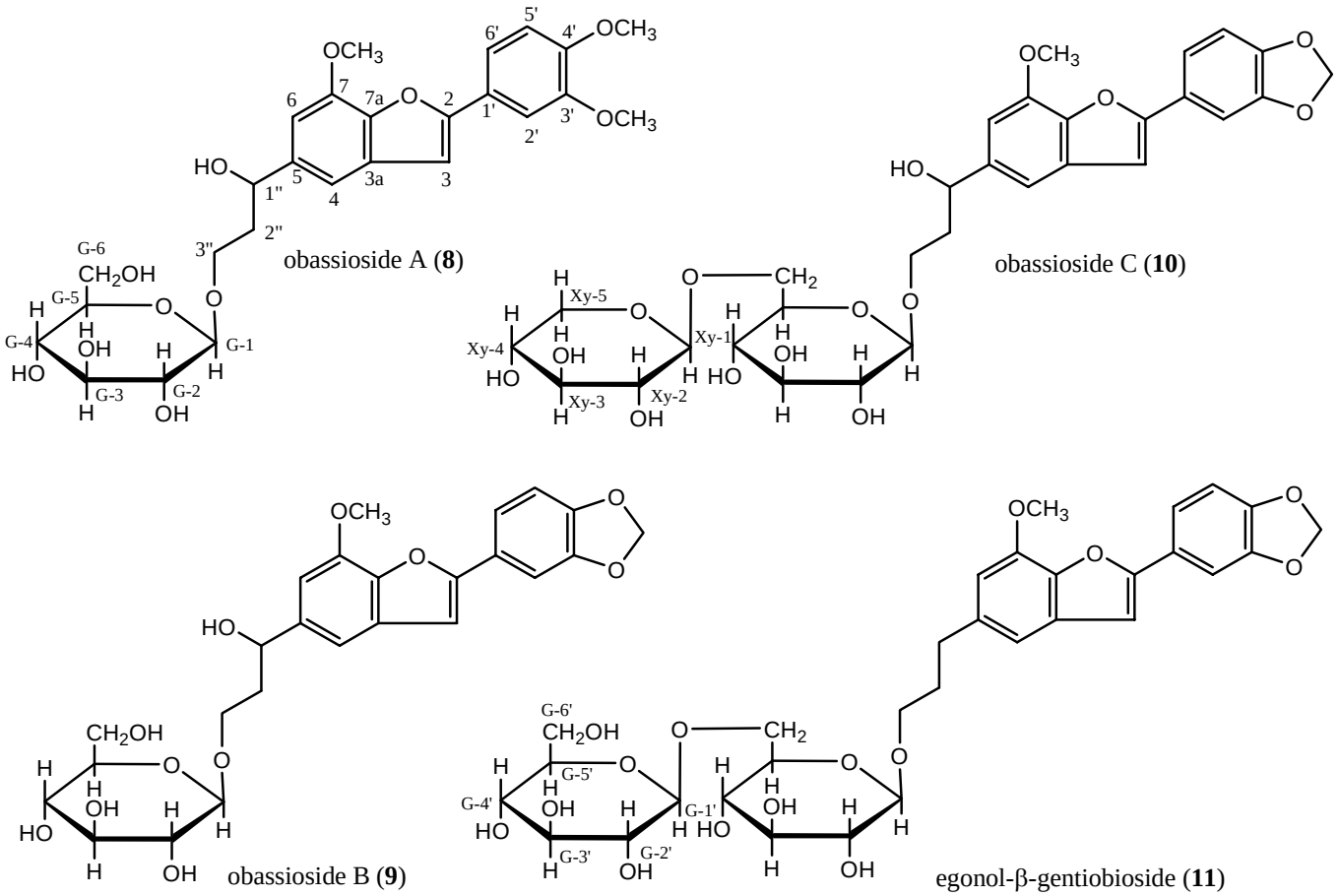


Figure 2 Constituents isolated from *Styrax obassia* bark

investigated,[2,4,6] we selected the bark of this plant as the target for chemical investigation in search of neolignans, which we regard as part of our long-term research concept project "*bioprospecting targeted for domestic natural resource*". This report refers to the isolation and structure elucidation of three new *nor*-neolignan glycosides from *S. obassia* bark.

The methanol extract of the bark of this plant was partitioned between water and organic solvents such as ethyl acetate and 1-butanol. The 1-butanol extract was subjected to a series of chromatographic separation to yield a known compound egonol-β-gentiobioside (**11**) that was reported from the seeds of *S.officinalis*[3] and *S. obassia*,[6] and three new compounds for which the names of obassiosides A (**8**), B (**9**) and C (**10**) were proposed.

Obassioside A (**8**) was obtained in optically active amorphous form, $[\alpha]_D$ -14.7 °. It has a molecular composition of $C_{26}H_{32}O_{11}$ as determined by the high-resolution FABMS spectrometry. The structure of this compound was assigned to **8** on the basis of the spectroscopic evidence as mentioned below. It exhibited UV absorption maxima at 216 and 312 nm, which is characteristic of the 2-phenylbenzofuran skeleton,[4] and an IR absorption at 3451 cm^{-1}, which is indicative of this compound being a polyhydroxy compound. The ^{1}H-NMR spectrum revealed the presence of AB (a pair of doublets with *J*=1.2 Hz at δ 6.92 and 7.17) and ABX (a doublet with *J*=8.2 Hz at δ 7.01, a double doublet with *J*=8.2 and 1.9 Hz at δ 7.44 and a doublet with *J*=1.9 Hz at δ 7.43) aromatic proton systems, an olefinic methine singlet at δ 7.00 and three methoxyl signals at δ 3.86, 3.91 and 4.03. The above spectroscopic data indicated that obassioside A was a 5-substituted-7-methoxy-2-(3,4-dimethoxyphenyl)benzofuran, which was also supported by the ^{13}C-NMR spectral data (Table I). The analysis of the rest of resonances was based on a combination of 2D-NMR techniques such as ^{1}H-^{1}H COSY, HMQC and HMBC spectra. The presence of glucopyranose was evident from resonances for five oxygenated methines at δ 3.23 (1H, dd, *J*=9.2 and 7.7 Hz), 3.26 (1H, m), 3.29 (1H, dd, *J*=8.8 and 8.8 Hz), 3.37 (1H, dd, *J*=9.2 and 8.8 Hz) and 4.26 (1H, d, *J*=7.7 Hz), which had HMQC correlations with oxygenated carbon signals at δ 75.14, 77.91, 71.70, 78.12 and 104.48, respectively, along with one oxygenated methylene proton signals at δ 3.66 (1H, dd, *J*=11.8 and 5.5 Hz) and 3.86 (1H, dd, *J*=11.8 and 2.2 Hz), which the HMQC spectrum indicated were directly attached to an oxygenated carbon of δ 62.76. The coupling constant (7.7 Hz) of a signal at δ 4.26 that was assigned to the anomeric proton clearly indicated that glucose had the β-configuration. The

Table I. ^{1}H- and ^{13}C-NMR Spectral data[a)] for Obassiosides A (8), B (9) and C (10).

	Obassioside A (8)		Obassioside B (9)		Obassioside C (10)	
	^{1}H (multiplicity, *J* Hz)	^{13}C	^{1}H (multiplicity, *J* Hz)	^{13}C	^{1}H (multiplicity, *J* Hz)	^{13}C
2	-	157.66	-	157.43		157.42
3	7.00 (s)	101.52	7.00 (s)	101.64	7.01 (s)	101.67
3a	-	132.31	-	132.20		132.20
4	7.17 (d, 1.2)	111.50	7.20 (d, 1.2)	111.51	7.21 (d, 1.1)	111.53
5	-	142.20	-	142.21		142.14
6	6.92 (d, 1.2)	105.98	6.96 (d, 1.2)	106.11*	6.96 (d, 1.1)	106.19*
7	-	146.29	-	146.28		146.27
7a	-	144.45	-	144.40		144.39
1'	-	124.94	-	125.99		126.00
2'	7.43 (d, 1.9)	109.53	7.36 (d, 1.6)	106.10*	7.37 (d, 1.6)	106.13*
3'	-	150.75	-	149.63	-	149.63
4'	-	151.13	-	149.54	-	149.54
5'	7.01 (d, 8.2)	113.10	6.93 (d, 8.0)	109.61	6.94 (d, 8.2)	109.62
6'	7.44 (dd, 8.2, 1.9)	119.08	7.44 (dd, 8.0, 1.6)	120.06	7.44 (dd, 8.2, 1.6)	120.07
1"	4.94 (m)	72.43	4.97 (m)	72.40	4.99 (m)	72.31
2"	2.02 (m) 2.11 (m)	40.50	2.06 (m) 2.15 (m)	40.46	2.06 (m) 2.13 (m)	40.51
3"	3.57 (m) 4.05 (m)	67.90	3.61 (m) 4.09 (m)	67.90	3.64 (m) 4.09 (m)	68.14
G-1	4.26 (d, 7.7)	104.48	4.30 (d, 7.7)	104.46	4.30 (d, 8.0)	104.55
G-2	3.23 (dd, 9.2, 7.7)	75.14	3.27 (dd, 9.0, 7.7)	75.11	3.2-3.3 (m)	75.02
G-3	3.37 (dd, 9.2, 8.8)	78.12	3.41 (dd, 9.0, 8.6)	78.10	3.40 (dd, 8.9, 7.8)	77.92
G-4	3.29 (dd, 8.8, 8.8)	71.70	3.34 (dd, 8.6, 8.6)	71.68	3.2-3.3 (m)	71.53
G-5	3.26 (m)	77.91	3.28 (m)	77.89	3.2-3.3 (m)	76.86
G-6	3.66 (dd, 11.8, 5.5) 3.86 (dd, 11.8, 2.2)	62.76	3.71 (dd, 11.8, 5.6) 3.90 (dd, 11.8, 2.2)	62.74	3.77 (dd, 11.4, 6.0) 4.14 (dd, 11.4, 2.1)	69.78
Xy-1	-	-	-	-	4.31 (d, 7.9)	105.44
Xy-2	-	-	-	-	3.2-3.3 (m)	74.82
Xy-3	-	-	-	-	3.47 (m)	77.62
Xy-4	-	-	-	-	3.52 (ddd, 5.5, 8.7, 10.1)	71.14
Xy-5	- -	- -	- -	- -	3.35 (m) 3.89 (dd, 5.5, 11.5)	66.89
7-OMe	4.03 (s)	56.60*	4.08 (s)	56.66	4.07 (s)	56.69
OCH_2O	-	-	6.03 (s)	102.76	6.04 (s)	102.77
3'-OMe	3.91 (s)	56.49*	-	-	-	-
4'-OMe	3.86 (s)	56.58*	-	-	-	-

a) δ ppm, measured in MeOH-d_4 with TMS as an internal standard (^{1}H, 600 MHz; ^{13}C 150 MHz).

* Figures in the same column are interchangeable.

^{13}C-NMR spectrum exhibited the presence of one methylene carbon signal at δ 40.50 that correlated with proton signals at δ 2.02 and 2.11, one oxygenated methylene carbon signal at δ 67.90 that correlated with proton signals at δ 3.57 and 4.05, and one oxygenated methine carbon signal at δ 72.43 that correlated with a proton signal at δ 4.94, respectively, in the HMQC spectrum. These results coupled with the ^{1}H-^{1}H COSY data readily assigned the structure of this moiety as ■-CH(O-■)-CH_2-CH_2-O-■. The HMBC spectrum indicated the occurrence of glucose at the 3''-position of the 1,3-oxypropanoid moiety (correlations: H-3''/G-C1 and G-H1/C-3''). The structure for obassioside A was unequivocally established as **8**, which was further substantiated by the HMBC correlations. Enzymic hydrolysis of **8** afforded the aglycon, the structure of which was confirmed on the spectroscopic basis as 5-(1,3-dihydoroxypropyl)-7-methoxy-2-(3,4-dimethoxyphenyl)benzofuran (**7**). Though it had an asymmetric carbon at the 1''-position, there were no suitable means available that enable its absolute stereochemical assignment. The sugar moiety of **8**, after acidic hydrolysis, was identified on HPLC with the authentic glucose. It is of interest to note is that the aglycone of obassioside A remains to be known in nature, and thus the name of *obassiol* (**7**) for the aglycone was proposed.

Obassioside B (**9**) was also obtained in optically active amorphous form, $[\alpha]_D$ -13.0 ^{o}. The molecular formula $C_{25}H_{28}O_{11}$ was determined by the high-resolution FABMS spectrometry. Both the ^{1}H- and ^{13}C-NMR spectra were closely similar to those of obassioside A (**8**) except that obassioside B (**9**) had resonance for a 2H singlet at δ 6.03 that was assignable to a methylenedioxy group in place of two methoxyls in obassioside A (**8**). The HMBC and HMQC analysis readily confirmed the structure for obassioside B as **9**. Enzymic hydrolysis of obassioside B yielded 5-(1,3-dihydoroxypropyl)-7-methoxy-2-(3,4-methylenedioxy)benzofuran (**5**), which was a known compound machicendiol (**5**) isolated from *Machillus glaucescens* (Lauraceae) and was identified with the authentic sample by comparison with its spectral data described in the literature.[9,10] This compound has not yet been obtained in the glycosidic form from *Machillus glaucescens*,[9,10] and its occurrence in *Styrax* species remains to be known. This is the first report of isolation from nature as the glycoside.

Obassioside C (**10**) was obtained in optically active amorphous form, $[\alpha]_D$ -17.3 ^{o}. As readily presumed by

the fact that this compound was obtained from more polar chromatographic fractions, it is apparent from its molecular formula $C_{30}H_{36}O_{15}$ deduced by HR FABMS that this compound is a disaccharide. Acidic hydrolysis afforded a mixture of two monosaccharides, which were identified with glucose and xylose by HPLC analysis. Enzymic hydrolysis of **10** afforded the same aglycone machicendiol (**5**)[9,10] as **9**. The close similarity of the ^{1}H- and ^{13}C-NMR spectra of both compounds as shown in Table I revealed that obassioside C (**10**) was a xyloside form of obassioside B (**9**). The glycosidic linkage in the disaccharide was determined to be at the 6'-position of glucose from the finding that a marked downfield shift by *ca* 7 ppm was observed with the 6'-methylene carbon signal (δ 69.78) of glucose as compared to the corresponding carbon signal (δ 62.74) in obassioside B. The structure **10** for obassioside C was consistent with results of the HMBC and HMQC analysis in every respect.

Egonol-β-gentiobioside (**11**) was isolated first from *S. officinalis* seeds[3] as glycoside mixtures, and its structure elucidation was described on its permethyl ether. Recently, the isolation of this compound was reported from *S. obassia* immature seeds.[6] It should be noted that all constituents isolated from fruits of the latter plant were either ester or glycoside forms of egonol (**4**). In this report we revealed the occurrence of 3''-hydroxy form of egonol glycosides in the bark of this species. A number of lignans were obtained from the bark of *S. japonica*,[11] the close botanical ally of *S. obassia*, but *nor*-neolignans have not been isolated yet. On the other hand, we have obtained not lignans but *nor*-neolignans as described in this report. This fact is of particular interest from the chemosystematic viewpoint. One of the two aglycones of glycosides obtained from *S. obassia* bark has also been found in Lauraceous plant *Machillus glaucescens*,[9,10] which has no relation to *Styrax* species from the systematic viewpoint. The disjunct distribution of characteristic secondary metabolites such as egonol (**4**) and its phytochemical allies is quite puzzling for phytochemists who endeavor to utilize chemotaxonomy as a tool for bioprospecting. This fact exemplifies the difficulty of integrating chemotaxonomy into morphology-based taxonomy.

We initiated this study with the intention of discovering kadsurenone-type neolignans from the genus *Styrax* as mentioned above. It was not indiscriminate but carefully elaborated search on the basis of chemosystematic accounts. Though the achievement of our research was far from satisfactory, we believe

that it would be materialized by extending the target for chemical investigation to the rest of the genus *Styrax* that consists of about one hundred species.

EXPERIMENTAL

General

All melting points were measured on a Yanagimoto melting point apparatus and are uncorrected. Spectral data were obtained using the following apparatus: ^{1}H- and ^{13}C-NMR spectra with a JEOL JNM ECP-600 (^{1}H, 600 MHz; ^{13}C, 150 MHz) spectrometer with tetramethylsilane (TMS) as internal standard; MS spectra (ms) and high-resolution mass spectra (HRMS) with a JEOL SX-102A mass spectrometer; IR spectra with a JASCO FT/IR-8000 infrared spectrometer; optical rotations with a JASCO DIP-370 polarimeter and UV spectra with a Shimadzu UV-260 spectrometer. Column chromatography was carried out with Wakogel C-200 or Merck Kieselgel 60, Sephadex LH-20 (Pharmacia, eluted with MeOH) and RP-8 reversed-phase silica gel (eluted with MeOH-H_2O), and TLC was conducted on a 0.25 mm pre-coated silica-gel plate (60F_{254}, Merck), and spots were detected by inspection under short (254 nm) or long (365 nm) wavelength UV lights, or by the colors developed with 10% H_2SO_4 spraying followed by heating on a hot plate.

Plant material

The bark of *S. obbasia* was collected from a blowdown tree cultivated at Medicinal Plant Garden, Teikyo University, Japan, in August 2003. A voucher specimen was on deposit at the Herbarium of Medicinal Plant Garden, Teikyo University.

Extraction and Isolation

The dried bark (708 g) of *S. obassia* was crushed, extracted with MeOH (4 L) three times at rt for 2 days and the combined extract was concentrated *in vacuo*. The MeOH extract (149.3 g) was dissolved in distilled water (3 L), and insolubles (9.5 g) were removed by filtration. The water layer was extracted successively with AcOEt and 1-BuOH, which on evaporation *in vacuo* yielded the AcOEt (38.0 g) and 1-BuOH (65.6 g) extracts, respectively. The 1-BuOH extract was chromatographed over SiO_2 (500 g), and

the column was eluted with the following solvent system: $CHCl_3$ (450 mL) $CHCl_3$-MeOH 19:1 (900 mL), 9:1 (900 mL), 85:15 (900 mL), 4:1 (900 mL), 3:1 (900 mL), 2:1 (900 mL), 3:2 (900 mL), MeOH (1.8 L). Fractions of 450 mL each were taken and nineteen fractions collected. Fraction 8 of this chromatographic separation, which was eluted with $CHCl_3$-MeOH 3:1, was repeatedly chromatographed over Sephadex LH-20, RP-8, and RP-18 and finally purified by preparative TLC to furnish obassiosides A (**8**) (820 mg) and B (**9**) (600 mg). Fraction 9 eluted with $CHCl_3$-MeOH 3:1 was also subjected to a series of column chromatography over Sephadex LH-20, RP-8, and RP-18 to furnish obassioside B (**9**) (970 mg) and a known compound egonol-β-gentiobioside (**11**) (246 mg). Fractions 12 and 13 were combined and purified in the same manner as stated above to give obassioside C (**10**) (1290 mg).

Obassioside A (8). Amorphous. $[\alpha]_D^{25}$ -14.7 ^{o} (*c* = 0.236, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3451 (OH), 2921, 1651, 1637, 1603, 1514, 1271, 1022. UV λ_{max}^{MeOH} nm (log ε): 216 (4.36), 312 (4.33). ^{1}H-NMR (600 MHz, MeOH-d_4): see Table I. ^{13}C-NMR (150 MHz, d_4-MeOH): see Table I. HRFABMS: $(M+Na)^+$ 543.1842 ($C_{26}H_{32}O_{11}Na$ requires 543.1858).

Obassioside B (9). Amorphous. $[\alpha]_D^{25}$ -13.0 ^{o} (*c* = 0.226, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3424 (OH), 2926, 1636, 1505, 1478, 1040. UV λ_{max}^{MeOH} nm (log ε): 216 (4.39), 301 (4.26), 316 (4.33). ^{1}H-NMR (600 MHz, MeOH-d_4): see Table I. ^{13}C-NMR (150 MHz, MeOH-d_4): see Table I. HRFABMS: $(M+Na)^+$ 527.1529 ($C_{25}H_{28}O_{11}Na$ requires 527.1552).

Obassioside C (10). Amorphous. $[\alpha]_D^{25}$ -17.3 ^{o} (*c* = 0.230, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3389 (OH), 2917, 1651, 1624, 1603, 1505, 1478, 1040. UV λ_{max}^{MeOH} nm (log ε): 217 (4.08), 301 (3.93), 316 (4.00). ^{1}H-NMR (600 MHz, MeOH-d_4): see Table I. ^{13}C-NMR (150 MHz, MeOH-d_4): see Table I. HRFABMS: $(M+Na)^+$ 659.1952 ($C_{30}H_{36}O_{15}Na$ requires 659.1945).

Egonol-β-gentiobioside (11). Colorless fine needles from MeOH, mp 209-210°C. IR ν_{max}^{KBr} cm^{-1}: 3424 (OH), 2932, 1636, 1622, 1507, 1476, 1040. UV λ_{max}^{MeOH} nm (log ε): 217 (4.42), 301 (4.31), 316 (4.37). ^{1}H-NMR (600 MHz, DMSO-d_6): δ 1.86 (2H, m, 2"-H_2), 2.71 (2H, m, 1"-H_2), 3.07 (7H, m), 3.27 (3H, m), 3.41 (1H, dd, *J*=11.1, 6.2 Hz), 3.48 (1H, dd, *J*=11.3, 5.5 Hz), 3.79 (1H, m), 3.93 (1H, dd, *J*=11.5, 1.6 Hz),

4.12 (1H, d, *J*=7.9 Hz), 4.21 (1H, d, *J*=7.7 Hz), 4.62 (1H, dd, *J*=5.5, 5.5 Hz), 4.95 (1H, d, *J*=4.7 Hz), 4.97 (1H, d, *J*=4.9 Hz), 4.99 (1H, d, *J*=4.9 Hz), 5.01 (1H, d, *J*=4.7 Hz), 5.03 (1H, d, *J*=5.5 Hz), 5.09 (1H, d, *J*=4.9 Hz), 6.08 (2H, s, OCH_2O), 6.78 (1H, d, *J*=1.4 Hz, 6-H), 7.00 (1H, d, *J*=1.4 Hz, 4-H), 7.03 (1H, d, *J*=8.2 Hz, 5'-H), 7.20 (1H, s, 3-H), 7.39 (1H, dd, *J*=8.0, 1.6 Hz, 6'-H), 7.41 (1H, d, *J*=1.6 Hz, 2'-H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ 31.53 (C-2''), 31.88 (C-1''), 55.86 (7-OMe), 60.32 (G-6'), 65.73 (C-3''), 67.90 (G-6), 68.41 (G-4 or G-4'), 69.64 (G-4' or G-4), 69.92 (G-5), 72.31 (G-5'), 73.38 (G-2 or G-2'), 73.47 (G-2' or G-2), 75.75 (G-3 or G-3'), 76.65 (G-3' or G-3), 101.17 (C-3), 101.52 (OCH_2O), 102.87 (G-1), 104.06 (G-1'), 105.03 (C-2'), 107.90 (C-6), 108.93 (C-5'), 112.27 (C-4), 118.80 (C-6'), 124.12 (C-1'), 130.52 (C-3a), 137.95 (C-5), 141.67 (C-7a), 144.39 (C-7), 147.87 (C-4' or C-3'), 148.03 (C-3' or C-4'), 155.19 (C-2).

Enzymic hydrolysis of glycosides by emulsin

The solution of obassioside A (**8**; 3 mg) in MeOH (200 μL) was added to the NaOAc-HOAc buffer solution (pH 5; 5mL) containing emulsin (5 mg), and the mixture was allowed to stand overnight at 37°C. The reaction mixture was extracted repeatedly with AcOEt, and the combined organic layer was evaporated to dryness. The residue was purified by preparative TLC to afford (-)-5-[(1,3-dihydroxy)propyl]-7-methoxy-2-(3,4-dimethoxyphenyl)benzofuran (**7**) (*obassiol*; 1.6 mg). Amorphous. $[\alpha]_D^{25}$ -9.8° (*c* = 0.34, MeOH). UV λ_{max}^{MeOH} nm (log ε): 216, 312. ^{1}H-NMR (600 MHz, MeOH-d_4): δ 1.96, 2.14 (2H each, m, 2''-H_2), 3.86 (3H, s, 4'-OMe), 3.90 (3H, s, 3'-OMe), 4.00 (2H, m, 3''-H_2), 4.04 (3H, s, 7-OMe), 4.96 (1H, dd, *J*=9.0, 4.2 Hz, 1''-H), 6.90 (1H, d, *J*=1.3 Hz, 6-H), 6.98 (1H, s, 3-H), 7.00 (1H, d, *J*=8.3 Hz, 5'-H), 7.15 (1H, d, *J*=1.3 Hz, 4-H), 7.42 (1H, d, *J*=1.9 Hz, 2'-H), 7.45 (1H, dd, *J*=8.3, 1.9 Hz, 6'-H). EIMS: *m*/*z* (rel. int., %) 358 (M^+, 100), 340 (12), 314 (72). The hydrolysis of both obassiosides B (**9**) and C (**10**) was also carried out in the same manner as stated above, and afforded the same aglycone, which was identified with *machicendiol* (**5**) in comparison with spectral data reported in the literature.[9,10]

Identification of sugar moiety

To a solution of obassioside A (**8**; 3 mg) in MeOH (2 mL) was added 10% H_2SO_4 (3 mL), and the mixture was on reflux at 100°C for 2 h. The reaction mixture was then cooled and extracted with AcOEt. The

aqueous layer was neutralized with Amberlite IRA-35 and evaporated *in vacuo* to dryness. Obassiosides B (**9**) and C (**10**) were also treated in the same manner for the identification of sugar moiety. Each of the residues obtained from acidic hydrolysis was subject to HPLC (EYELA PLC-5, Tokyo Rikakikai Co., Ltd., column: Asahipak NH_2P-50 4E, H_2O-CH_3CN=20:80, 1.0m*l*/min, 30 ℃) analysis for the identification of sugars of each glycoside, which was determined by RI detection by comparison with authentic sugars: glucose (t_R, 13.4 min) and xylose (t_R, 8.9 min).

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