Staphylionosides A—K: Megastigmane Glucosides from the Leaves of *Staphylea bumalda* DC.

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Chemical investigation of leaves of *Staphylea bumalda* DC., collected in the suburbs of Hiroshima City, afforded 11 new megastigmane glucosides, named staphylionosides A—K (3—13), along with two known megastigmane glucosides (1, 2). The relative structures were elucidated from spectroscopic evidence, and the absolute structures of the aglycones were determined by means of the combination of β-D-glucosylation-induced shift-trends and the modified Mosher's method.

Key words *Staphylea bumalda*; Staphyleaceae; megastigmane glucoside; staphylionoside; modified Mosher's method

Megastimgane and its glycosides are a currently expanding class of compounds. The Staphyleaceae comprise seven genera and about 50 species throughout the world, almost all of which are found in temperate zone of the Northern Hemisphere. In Japan, three species from different genera of the Staphylaceae grow in moderate and subtropical areas.[1] In previous studies, the chemical constituents of *Euscaphis japonica* (THUNB.) KANITZ[2] and *Turpinia ternata* NAKAI[3] were investigated. The third species growing in Japan is *Staphylea bumalda* DC. *S. bumalda* can be found throughout eastern Asia, especially in China, Japan and Korea. It is a deciduous tree growing to about three to five meters high, and blooms in May to June. A decoction of its fruit is used as a cough remedy and its fresh roots are used for blood refreshment after delivery.[4] The dried fruit is also used as a folk anti-diarrheal medicine as. So far, only one chromone glucoside, staphylin, has been reported as a constitutent of *S. bumalda*.[5] In this study, *S. bumalda* collected in the suburbs of Hiroshima City was phytochemically investigated, 11 new and two known megastigmane glucosides being obtained.

Results and Discussion

Air-dried leaves (5.71 kg) of *S. bumalda* were extracted with MeOH (15 l×3) and then the MeOH extract was concentrated to 3 l. After washing with *n*-hexane (3 l), the MeOH extract was concentrated to a viscous gum and then suspended in H_2O. The suspension was extracted with EtOAc (3 l) and *n*-BuOH (3 l) successively to give EtOAc- (325 g) and *n*-BuOH-soluble (133 g) fractions, respectively. The *n*-BuOH-soluble fraction was separated by various kinds of column chromatography (CC) on a highly porous synthetic polymer (Diaion HP-20), normal and reversed-phase silica gel, and droplet counter-current chromatography (DCCC), and preparative HPLC to give 13 compounds (**1**—**13**).

Compound **1**, $[\alpha]_D$ −63.7°, was identified with icariside B_2, previously isolated from *Epimedium gradiflorum* var. *grandiflorum*, by spectroscopic analysis.[6] The NMR spectra of compound **2** were essentially the same as those reported for (3*S*,5*R*,6*R*,9*S*,7*E*)-megastigman-7-ene-3,5,6,9-tetrol 9-*O*-β-D-glucopyranoside (**2′**) isolated from *Glochidion zeylanicum*.[7] Since stereochemistry on the ring must be determined independently,[7] compound **2** was hydrolyzed with an enzyme and the modified Mosher's method[8] was applied to the aglycone. As a result, compound **2** was proved to be the same as **2′**.

Staphylionoside A (**3**), $[\alpha]_D$ −110.7°, was isolated as an amorphous powder and its elemental composition was determined to be $C_{19}H_{30}O_8$ by high-resolution (HR)-FAB-MS. The

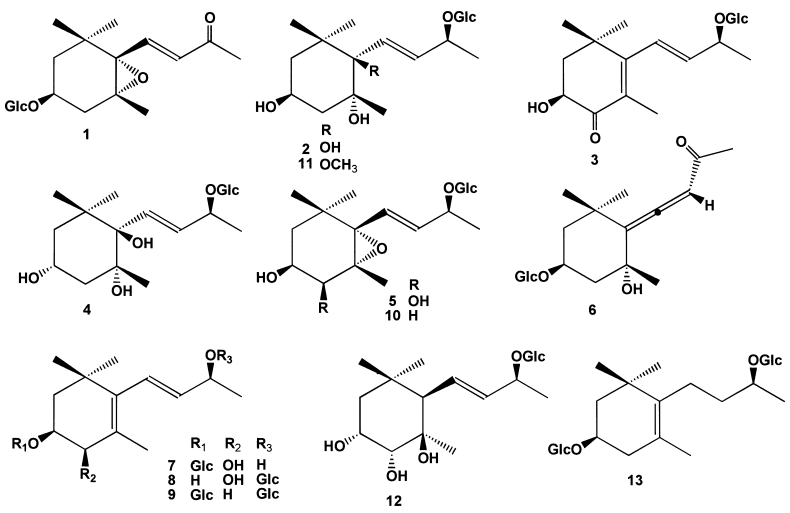

Fig. 1. Structures

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Table 1. ^{13}C-NMR Data for Staphylionosides A—J (**3**—**12**) (CD_3OD, 100 MHz)

Carbon No.	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	38.6	35.2	35.3	37.0	37.9	37.7	37.6	35.9	43.0	35.5	38.8
2	47.0	44.2	40.5	46.7	40.1	41.8	47.2	47.9	46.4	43.3	47.6
3	70.5	69.0	66.7	72.7	76.2	68.0	73.2	64.6	65.4	67.0	73.4
4	201.9	41.8	73.3	48.2	70.2	72.2	39.8	41.6	46.6	78.4	39.9
5	129.0	77.5	69.7	72.4	128.0	129.4	127.1	68.1	78.5	76.1	125.2
6	162.4	80.3	71.6	120.2	143.0	142.2	138.2	71.1	86.4	53.4	138.7
7	129.2	136.3	129.6	200.9	126.7	131.0	131.4	129.6	129.9	132.2	25.1
8	140.0	132.4	136.2	101.2	140.7	137.5	136.8	136.1	137.1	137.0	38.0
9	75.2	75.7	74.7	211.5	69.4	75.6	75.7	74.8	76.3	75.6	77.9
10	22.3	27.6	22.4	26.6	23.9	22.4	22.4	22.5	22.6	22.6	21.8
11	26.0	27.6	29.8	29.5	30.3	30.7	21.8	30.1	28.3	23.6	28.9
12	31.3	28.9	24.9	32.3	27.8	27.8	28.7	25.1	26.5	33.1	30.0
13	14.0	22.6	17.8	30.9	19.9	20.2	31.0	20.7	28.7	28.6	20.2
–OMe									55.6		
1′	101.6	100.5	101.4	102.8	102.7	101.7	102.5	101.4	101.1	100.9	102.4
2′	75.1	75.1	75.1	75.2	75.4	75.1	75.1[a)]	75.1	75.1	75.1	75.3[d)]
3′	78.4	78.3	78.1	78.2	78.1	78.1	78.0	78.3	78.3	77.9	78.2[e)]
4′	71.9	71.8	71.8	71.7	71.7	71.9	71.7[b)]	71.8	71.8	71.8	71.8
5′	78.3	77.9	78.3	77.9	78.1	78.4	78.0	78.1	78.4	78.3	77.9
6′	63.0	62.8	62.9	62.8	62.8	62.9	62.8[c)]	62.9	62.9	62.6	62.9
1″							100.9				103.9
2″							75.2[a)]				75.4[d)]
3″							78.0				78.3[e)]
4″							71.9[b)]				71.8
5″							78.0				77.9
6″							62.9[c)]				62.9

a—*e*) The same superscripts may be interchangeable.

^{13}C-NMR spectrum together with the ^{1}H-NMR spectrum showed the presence of six signals assignable to *β*-glucopyranose. The remaining 13 signals comprised those of three singlet methyls, one of which must be on a double bond (δ_H 1.85, 3H, d, J=1 Hz), one doublet methyl, one methylene, two methines with an oxygen substituent, two quaternary carbons, and a disubstituted *trans* double bond [δ_H 5.66 (d, J=16, 7 Hz) and 6.39 (br d, J=16 Hz)], and a tetrasubstituted double bond. One of the quaternary carbons was expected to possess a ketonic oxygen functional group from the highly deshielded chemical shift (δ_C 201.9) in the ^{13}C-NMR spectrum. From the above evidence, the structure of **3** was assumed to be a megastigmane glucoside with two hydroxyl and one ketonic functional groups. The disubstituted double bond must be located between C-7 and C-8, and the tetrasubstituted one between C-4 and C-6. Judging from the UV absorption band at 262 nm, the ketone functional group was in a conjugated system, such as at the C-4 position. This was confirmed by heteronuclear multiple bond correlation (HMBC) spectroscopy, in which δ_H 1.85 (H_3-13) crossed δ_C 201.9 (C-4). Finally, based on the observation of HMBC cross peaks between geminal methyls at the 1-position (δ_H 1.18, 1.31) and a methylene carbon (δ_C 47.0), the planar structure of the aglycone moiety was assigned as megastigma-5,7-diene-3,9-dihydroxy-4-one. The position of the glucosidic linkage was also determined to be the hydroxyl group at the 9-position from the HMBC spectrum, in which δ_H 4.60 (H-9) crossed the anomeric carbon signal at δ_C 101.6 (C-1′). To determine the absolute configuration of the two chiral centers, **3** was enzymatically hydrolyzed to give D-glucose ([α]$_D$ +39.8°) and the corresponding diol (**3a**), which was then converted to esters with (*R*)- and (*S*)-*α*-methoxy-*α*-trifluoromethylphenylacetic acids (MTPA) (**3b**, **c**, respectively).[8] Figure 2 shows that the absolute configurations at the 3- and 9-positions are the same and to be *S*. Therefore the structure of staphylionoside A was elucidated to be (3*S*,9*S*,5*Z*,7*E*)-megastigma-5,7-diene-3,9-dihydroxy-4-one 9-*O*-*β*-D-glucopyranoside (**3**).

Staphylionoside B (**4**), [α]$_D$ −30.0°, was isolated as an amorphous powder and its elemental composition was determined to be $C_{19}H_{34}O_9$ by HR-FAB-MS. The ^{13}C-NMR spectrum together with the ^{1}H-NMR spectrum showed the presence of six signals assignable to *β*-glucopyranose. The remaining 13 carbon signals comprised those of three singlet methyls, one doublet methyl, two methylenes, two methines with an oxygen substituent, three quaternary carbons, two of which were assumed to bear hydroxyl groups, and a disubstituted *trans* double bond [δ_H 5.60 (dd, J=16, 8 Hz) and 6.23 (d, J=16 Hz)]. Close inspection on two-dimensional NMR enabled to establish the planar structure to be megastigman-7-ene-3,5,6,9-tetrol 9-*O*-*β*-glucopyranoside. In the ^{13}C-NMR spectrum, broadening of carbon signals assignable to those for a six-membered ring [δ_C 35.2, 44.2, 69.0, 41.8, 77.5, 80.3 (C-1 to C-6)] was observed. In the ^{1}H-NMR spectrum, although the H-3 signal resonating at δ_H 4.08 appeared as a multiplet, from the coupling patterns of adjacent well-resolved proton signals, the coupling constant values of $J_{2ax\text{-}3}$, $J_{2eq\text{-}3}$, $J_{4ax\text{-}3}$ and $J_{4eq\text{-}3}$ were calculated to be 4 Hz, 5 Hz, 4 Hz and 5 Hz, respectively. Therefore, the hydroxyl group at the 3-position must have the axial orientation. Regarding the megastigmanes reported so far, this is the second example in which the hydroxyl group at the C-3 position must be in the axial orientation in the megastigmane skeleton.[9] The phase sensitive (PH)-NOESY spectrum indicated that the methyl group at the 5-position and the side chain at the 6-position were in the equatorial orientation (Fig. 3a). The hydroxyl

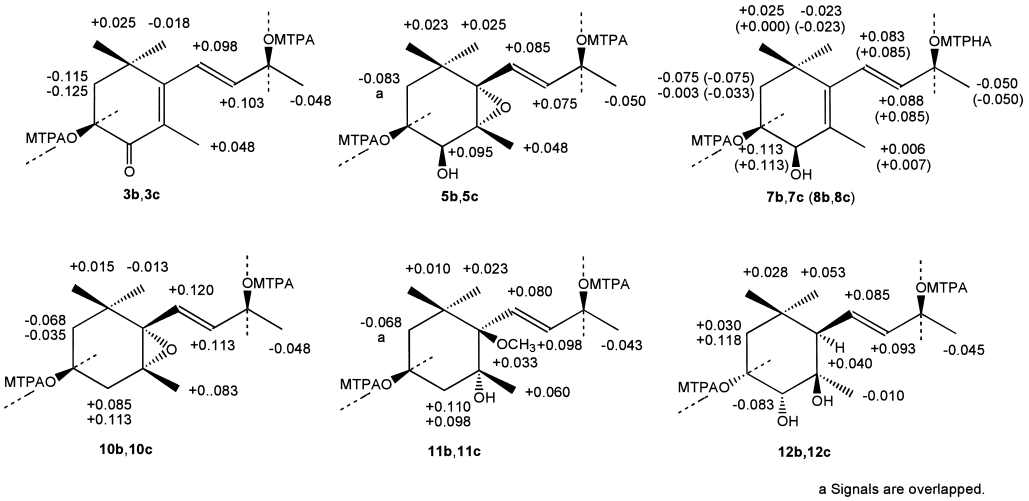

Fig. 2. Results with the Modified Mosher's Method ($\Delta\delta_S - \delta_R$)

substituent on the 3-position, together with another hydroxyl group at C-5 and an axial methyl group at the 1-position exhibited a 1,3-diaxial relationship, and this may have caused perturbation of the ring system due to steric hindrance and that the carbon signals were broadened. After removal of the sugar by enzymatic hydrolysis, the aglycone was derivatized to the corresponding MTPA esters. The $\Delta\delta$ values of MTPA esters are shown in Fig. 3b. It is known that since not an "irregular" arrangement, but opposite signs of the $\Delta\delta$ values in MTPA esters of 3α (axial)-cholesterol were observed to corresponding protons of MTPA esters of 3β (equatorial)-cholesterol,[8] the absolute configurations at the 3-position of cholesterols can be explored. Therefore, the MTPA esters as to the axial hydroxyl group at the 3-position of **4** would show the opposite signs of $\Delta\delta$ values to those for the equatorial hydroxyl group (Fig. 3b). Thus, the structure of **4** was established to be (3*R*,5*R*,6*R*,9*S*,7*E*)-megastigman-7-ene-3,5,6,9-tetrol 9-*O*-β-D-glucopyranoside.

Staphylionoside C (**5**), $[\alpha]_D$ −90.0°, was isolated as an amorphous powder and its elemental composition was determined to be $C_{19}H_{32}O_9$ by HR-FAB-MS. The ^{13}C-NMR spectrum together with the ^{1}H-NMR spectrum showed the presence of six signals assignable to β-glucopyranose. The remaining 13 carbon signals comprised those of three singlet methyls, one doublet methyl, one methylene, three methines with an oxygen substituent, three quaternary carbons, two of which were assumed to bear oxygen atoms, and a disubstituted *trans* double bond [δ_H 5.58 (d, J=16, 7 Hz) and 6.04 (dd, J=16, 1 Hz)]. The three degrees of unsaturation of the aglycone and the two carbon signals without hydrogen at δ_C 69.7 and 71.6 suggested that an epoxy ring is present between these carbons. The two hydroxyl groups on the ring were suggested to be in vicinal positions by the HH-COSY spectrum, and one (δ_H 3.88) of the two hydroxyl groups was presumed to be located at the 4-position, since HMBC correlation was observed between δ_H 3.88 and δ_C 17.8 (C-13). The relative stereochemistry of the substituents on the ring was determined by PH-NOESY and then their absolute configurations were also assigned by means of the modified Mosher's method, as shown in Fig. 2. Thus the structure of **5** was elucidated to be (3*S*,4*S*,5*R*,6*S*,9*S*,7*E*)-megastigman-7-ene-5,6-epoxy-3,4,9-triol 9-*O*-β-D-glucopyranoside.

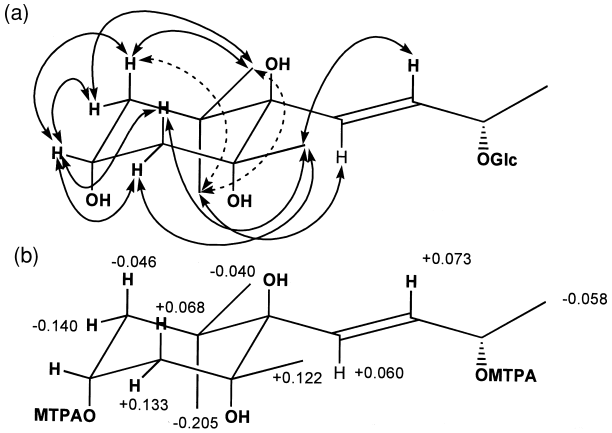

Fig. 3. (a) Diagnostic ^{1}H–^{1}H COSY and PH-NOESY Correlations

Dotted lines denote ^{1}H–^{1}H COSY correlations and dotted lines PH-NOESY correlations.

(b) Results for **4** with the Modified Mosher's Method

Staphylionoside D (**6**), $[\alpha]_D$ −60.8°, was also isolated as an amorphous powder and its elemental composition was determined to be $C_{19}H_{30}O_8$ by HR-FAB-MS. The UV spectrum exhibited an absorption maximum at 233 nm and the ^{13}C-NMR spectrum showed signals assignable to β-glucopyranose, and the remaining 13 signals included a typical allenic feature [δ_C 120.2 (s), 200.9 (s), 101.2 (d), 211.5 (s)], which has also been found for the side chain of icariside B_1 (**6′**).[6] Although the NMR data of the ring system were essentially superimposable on those of icariside B_1, the allenic carbon signal of C-7 and C-10 methyl signal appeared at higher field than those of **6′**.[10] Therefore, the structure of **6** was concluded to be an epimer of **6′** at the 7-position.

Staphylionosides E (**7**), $[\alpha]_D$ −99.0°, and F (**8**), $[\alpha]_D$ −118.1°, were each isolated as amorphous powders and their elemental compositions were determined to be the same as $C_{19}H_{32}O_8$ on HR-FAB-MS. The UV spectra showed an absorption maximum at 226 nm, and from the ^{13}C-NMR spectrum, **7** and **8** were found to be derivatives of megastigmane with two double bonds. One of the double bonds with four substituents must be located between C-5 and 6, and the other one in *trans* between C-7 and 8. Two-dimensional NMR spectroscopy revealed that two vicinal hydroxyl groups

were located at the C-3 and 4 positions, and the remaining one at the C-9 position. This functionalization of these compounds was the same as that of plucheoside B (**7′**) isolated from *Pluchea indica*,[11] in which two hydroxyl groups on the ring system have the *cis* configuration. The hydroxyl group at the 3-position of **7** carried β-glucopyranose as in the case of **7′**. Compound **7** was enzymatically hydrolyzed and the absolute structure of the aglycone (**7a**) was determined by the modified Mosher's method. Finally, the structure of staphylionosides E (**7**) was elucidated to be (3*S*,4*R*,9*S*,5*Z*,7*E*)-megastigma-5,7-diene-3,4,9-triol 3-*O*-β-D-glucopyranoside. Staphylionoside F (**8**) was assumed to be a positional isomer as to the sugar linkage. From the ^{13}C-NMR data, it was clarified that the sugar moiety was attached to the hydroxyl group at the 9-position. The aglycone was proved to have the same absolute configuration as that of **7** from the spectroscopic evidence regarding MTPA esters. Thus, the structure of **8** was elucidated to be (3*S*,4*R*,9*S*,5*Z*,7*E*)-megastigma-5,7-diene-3,4,9-triol 9-*O*-β-D-glucopyranoside. The agylcone of plucheoside B (**7′**) probably have the same absolute structure as those of **7** and **8** judging from similar specific optical rotation value ($[\alpha]_D$ −101.0°) of **7a** to that ($[\alpha]_D$ −116.0°) of the aglycone of **7′**.

Staphylionoside G (**9**) was assumed to be a megastigmame diglucoside from the NMR data, two anomeric proton [δ_H 4.40 (1H, d, J=8 Hz) and 4.43 (1H, d, J=8 Hz)] and carbon (δ_C 102.5, 100.9) signals, respectively, being observed. From the ^{13}C-NMR data together with the ^{1}H-NMR spectrum, one disubstituted and one tetrasubstituted double bonds, two methylenes, two methines with an oxygen function, and one quaternary carbon atom were shown to be present in the skeleton. Two singlet methyl, one doublet methyl and a broadened singlet methyl signals were also observed. Thus, **9** was expected to be a similar compound to platanionoside B isolated from *Alangium platanifolium* var. *platanifolium*.[12] Since only the ^{13}C-NMR chemical shifts at the 8, 9 and 10-positions were different between these two compounds, the structure of **9** was concluded to be an epimer of platanionoside B at the 9-position.

On close inspection of the physical data of staphylionoside H (**10**), it was presumed to be a similar compound to sammangaoside A isolated from *Clerodendron inerme*.[13] However, the absolute configuration at the 3-position of sammangaoside is not known. Then, compound **10** was enzymatically hydrolyzed and the configuration at the 3-position was determined to be *S* by the modified Mosher's method. Thus, the structure was elucidated to be (3*S*,5*R*,6*R*,9*S*,7*E*)-megastigman-5,6-epoxy-7-ene-3,9-diol 9-*O*-β-D-glucopyranoside.

Staphylionoside I (**11**), $[\alpha]_D$ −34.4°, was isolated as an amorphous powder and its elemental composition was determined to be $C_{20}H_{36}O_9$ by HR-FAB-MS. The ^{1}H- and ^{13}C-NMR spectra indicated the presence of one β-glucopyranose unit and a megastigmane skeleton as an aglycone. However, one more carbon signal, δ_C 55.6 with δ_H 3.36 (3H, s), namely a methoxyl group, was observed. This is in accordance with the results of mass spectrometry. On inspection of the HMBC and PH-NOESY spectra, the methoxyl group was found to be at the 6-position in the equatorial orientation and the hydroxyl group at the 5-position was in the axial orientation. The absolute structure of the aglycone was similarly elucidated by the modified Mosher's method. As a result, the configurations of C-3 and 9 were found to be the same as those of staphylionoside H (**10**), and the hydroxyl group at the C-5 position was also in the same orientation as that of the epoxy ring of **10**. Staphylionoside I (**11**) may be an artifact formed during extraction and isolation, and probably derived from staphylionoside H (**10**) through opening of the epoxy ring on the attack of the methoxyl anion at the 6-position. Although the isolation of many megastigmanes with an epoxy ring has been reported, this is the first time one with a methoxyl group has been isolated during a phytochemical investigation, even as an artifact.

Staphylionoside J (**12**), $[\alpha]_D$ −37.6°, was isolated as an amorphous powder and its elemental composition was determined to be $C_{19}H_{34}O_9$ by HR-FAB-MS. The ^{1}H- and ^{13}C-NMR spectra indicated that staphylionoside J was a megastigmane glucoside with a double bond between C-7 and 8, and four hydroxyl groups. From the ^{1}H–^{1}H COSY and HMBC spectra, three hydroxyl groups were found to be located in series from the 3 to 5-positions. The coupling constant of H-3 and 4 (4 Hz) indicated that these protons were in an axial and equatorial relationship, and the coupling pattern of H-3 (ddd, J=13, 4, 3 Hz) confirmed that H-3 was in the axial and H-4 in the equatorial orientation. Finally, the PH-NOESY spectrum showed that both the side chain and the C-13 methyl group were in the equatorial orientation. The absolute configuration at the 9-position was expected to be *S* from the glucose-induced shift-trend and that of the ring system was determined by the modified Mosher's method. The structure of staphylionoside J was elucidated to be (3*R*,4*R*,5*R*,6*R*,9*S*,7*E*)-megastigman-7-ene-3,4,5,9-tetraol 9-*O*-β-D-glucopyranoside.

Staphylionoside K (**13**) was assumed to be a megastigmame diglucoside from the NMR data, two anomeric proton [δ_H 4.34 (1H, d, J=8 Hz) and 4.44 (1H, d, J=8 Hz)] and carbon (δ_C 102.4, 103.9) signals, respectively, being observed. From the ^{13}C-NMR data together with the ^{1}H-NMR spectrum, one tetrasubstituted double bond, four methylenes, two methines with an oxygen function, and one quaternary carbon atom were shown to be present in the skeleton. Two singlet methyl, one doublet methyl and a broadened singlet methyl signals were also observed. Thus, **13** was expected to be a similar compound to linarionoside C (**13′**) isolated from *Linaria japonica*.[14] Although the stereochemistry of the ring portion was proved to be the same as that of **13′**, that of the side chain must be opposite to that of **13′** from the glucosylation-induced shift-trend. Therefore, the structure of staphylionoside K (**13**) was elucidated to be (3*S*,9*S*)-megastigman-5-ene-3,9-diol 3,9-di-*O*-β-D-glucopyranoside

Experimental

General Experimental Procedures A highly porous synthetic resin (Diaion HP-20) was purchased from Mitsubishi Kagaku (Tokyo, Japan). Silica gel column chromatography (CC) was performed on silica gel 60 (E. Merck, Darmstadt, Germany), and reversed-phase [octadecyl silica gel (ODS)] open CC (RPCC) on Cosmosil 75C_{18}-OPN (Nacalai Tesque, Kyoto) [Φ=50 mm, L=25 cm, linear gradient: MeOH–H_2O (1 : 9, 1 l)→(7 : 3, 1 l), fractions of 10 g being collected]. Droplet counter-current chromatography (DCCC) (Tokyo Rikakikai, Tokyo, Japan) was equipped with 500 glass columns (Φ=2 mm, L=40 cm), the lower and upper layers of a solvent mixture of $CHCl_3$–MeOH–H_2O–*n*-PrOH (9 : 12 : 8 : 2) being used as the stationary and mobile phases, respectively. Five-gram fractions were collected and numbered according to their order of elution with the mobile phase. HPLC was performed on ODS (Inertsil; GL Science, Tokyo, Japan; Φ=20 mm, L=250 mm), and the eluate was monitored with a UV detector at 254 nm

and a refractive index monitor. β-D-Glucosidase (emulsin) was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). The (*R*)-(+)- and (*S*)-(−)-MTPAs were from Nacalai Tesque.

Optical rotations were measured on a Union Giken PM-101 digital polarimeter. IR spectra were measured on a Horiba FT-710 Fourier transform infrared spectrophotometer and UV spectra on a JASCO V-520 UV/VIS spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were taken on a JEOL JNM α-400 spectrometer at 400 and 100 MHz, respectively, with tetramethylsilane (TMS) as an internal standard. Negative- and positive-ion HR-FAB-MS were taken on a JEOL JMS SX-102 spectrometer.

Plant Material Leaves of *Staphylea bumalda* DC. (Staphyleaceae) were collected in the suburbs of Hiroshima City, Japan, in June 2000, and a voucher specimen was deposited in the Herbarium of the Department of Pharmacognosy, Division of Medicinal Chemistry, Graduate School of Biomedical Sciences, Hiroshima University (00-SB-Hiroshima-0618).

Extraction and Fractionation The air-dried leaves of *S. bumalda* (5.71 kg) were extracted with MeOH (45 l) three times. The MeOH extract was concentrated to 3.0 l and then 150 ml of H_2O was added to make a 95% aqueous solution. This solution was washed with 3.0 l of *n*-hexane (74.4 g) and then the methanolic layer was concentrated to a viscous gum. The gummy residue was suspended in 3.0 l of H_2O, and then extracted with 3.0 l each of EtOAc and *n*-BuOH, successively, to afford 325 and 133 g of EtOAc- and *n*-BuOH-soluble fractions, respectively. The remaining H_2O layer was concentrated to furnish a H_2O-soluble fraction (323 g). A portion of the *n*-BuOH-soluble fraction (130 g) was subjected to highly porous synthetic resin (Diaion HP-20) CC (Mitsubishi Chemical Co., Ltd.; Φ=80 mm, L=80 cm), using H_2O–MeOH (4 : 1, 8 l), (2 : 3, 6 l), (3 : 2, 6 l), and (1 : 4, 6 l), and MeOH (3 l), 500 ml fractions were collected. The residue (12.3 g in fractions 9—11) of the 40% MeOH eluate obtained on HP-20 CC was subjected to silica gel (300 g) CC with increasing amounts of MeOH in $CHCl_3$ [$CHCl_3$ (2 l), and $CHCl_3$–MeOH (99 : 1, 3 l), (39 : 1, 3 l), (19 : 1, 3 l), (37 : 3, 3 l), (9 : 1, 3 l), (7 : 1, 3 l), (17 : 3, 3 l), (33 : 7, 3 l), (4 : 1, 3 l), (3 : 1, 3 l) and (7 : 3, 3 l)], and $CHCl_3$–MeOH–H_2O (70 : 30 : 4, 3 l), 500 ml fractions were collected. The residue (1.2 g in fractions 21—29) of the 5—10% MeOH eluate obtained on silica gel CC was subjected to RPCC and then the residue (194 mg in fractions 110—125) was purified by DCCC to give a compound **3**-enriched fraction, which was purified by HPLC to give 6.1 mg of pure **3**. The residue (1.80 g in fractions 30—36) of the 10—12.5% MeOH eluate obtained on silica gel CC was subjected to RPCC. The residues (227 mg in fractions 61—68, 109 mg in fractions 96—100 and 161 mg in fractions 101—110) were purified by DCCC to give compounds **6** (58.0 mg) from the first, **7** (51.0 mg) and **8** (8.0 mg) from the second, and **9** (5.2 mg) from the third residue. The residue (0.98 g in fractions 37—40) of the 12.5% MeOH eluate obtained on silica gel CC was subjected to RPCC, DCCC and HPLC to give compounds **10** (12.0 mg), **5** (7.2 mg), and **1** (6.1 mg). The residue (1.86 g in fractions 41—45) of the 15—17.5% MeOH eluate obtained on silica gel CC was subjected to RPCC, DCCC and HPLC to give **4** (45.2 mg), **11** (21.9 mg), **2** (18.0 mg), and **12** (8.0 mg).

The residue (24.0 g in fractions 12—15) of the 40—60% MeOH eluate obtained on Diaion HP-20 CC was subjected to silica gel (500 g) CC with increasing amounts of MeOH in $CHCl_3$ [$CHCl_3$ (2.0 l), and $CHCl_3$–MeOH [(99 : 1, 3 l), (39 : 1, 3 l), (19 : 1, 3 l), (37 : 3, 3 l), (9 : 1, 3 l), (7 : 1, 3 l), (17 : 3, 3 l), (7 : 3, 3 l), (4 : 1, 3 l), (3 : 1, 3 l) and (7 : 3, 3 l)], and $CHCl_3$–MeOH–H_2O (70 : 30 : 4, 3 l), 500 ml fractions were collected. The residue (1.67 g in fractions 29—37) of the 10—12.5% MeOH eluate was subjected to RPCC, DCCC and HPLC to give 7.3 mg of compound **13**.

Staphylionoside A (**3**): Amorphous powder; $[\alpha]_D^{26}$ −110.7° (c=0.41, MeOH). IR ν_{max} (film) cm^{-1}: 3395, 2967, 2938, 2870, 1669, 1452, 1370, 1311, 1158, 1075, 1036; UV λ_{max} (MeOH) nm (log ε): 262 (3.89); ^{1}H-NMR (CD_3OD) δ: 1.18 (3H, s, H_3-12), 1.31 (3H, s, H_3-11), 1.35 (3H, d, J=7 Hz, H_3-10), 1.81 (1H, dd, J=13, 13 Hz, H-2ax), 1.85 (3H, d, J=1 Hz, H_3-13), 2.07 (1H, dd, J=13, 6 Hz, H-2eq), 3.22 (1H, dd, J=9, 8 Hz, H-2′), 3.23 (1H, m, H-5′), 3.27 (1H, dd, J=9, 9 Hz, H-4′), 3.32 (1H, dd, J=9, 9 Hz, H-3′), 3.66 (1H, dd, J=12, 6 Hz, H-6′a), 3.88 (1H, dd, J=12, 2 Hz, H-6′b), 4.33 (1H, dd, J=13, 4 Hz, H-3), 4.36 (1H, d, J=8 Hz, H-1′), 4.60 (1H, qdd, J=7, 7, 1 Hz, H-9), 5.66 (1H, dd, J=16, 7 Hz, H-8), 6.39 (1H, br d, J=16 Hz, H-7); ^{13}C-NMR (CD_3OD): Table 1; HR-FAB-MS (negative-ion mode) m/z: 385.1875 [M−H]$^-$ (Calcd for $C_{19}H_{29}O_8$: 385.1862).

Staphylionoside B (**4**): Amorphous powder; $[\alpha]_D^{26}$ −30.0° (c=0.80, MeOH). IR ν_{max} (film) cm^{-1}: 3395, 2971, 2928, 1451, 1373, 1268, 1159, 1077, 1038; ^{1}H-NMR (CD_3OD) δ: 0.91 (3H, s, H_3-11), 1.16 (3H, s, H_3-12), 1.19 (3H, s, H_3-13), 1.31 (3H, d, J=6 Hz, H_3-10), 1.57 (1H, ddd, J=14, 5, 2 Hz, H-2eq), 1.73 (1H, ddd, J=14, 5, 2 Hz, H-4eq), 1.80 (1H, dd, J=14, 4 Hz, H-2ax), 2.05 (1H, dd, J=14, 4 Hz, H-4ax), 3.66 (1H, dd, J=12, 6 Hz, H-6′a), 3.87 (1H, dd, J=12, 2 Hz, H-6′b), 4.08 (1H, m, H-3), 4.40 (1H, d, J=8 Hz, H-1′), 5.52 (1H, dq, J=8, 6 Hz, H-9), 5.60 (1H, dd, J=16, 8 Hz, H-8), 6.23 (1H, d, J=16 Hz, H-7); ^{13}C-NMR (CD_3OD): Table 1; HR-FAB-MS (negative-ion mode) m/z: 405.2108 [M−H]$^-$ (Calcd for $C_{19}H_{33}O_9$: 405.2125).

Staphylionoside C (**5**): Amorphous powder, $[\alpha]_D^{25}$ −90.0° (c=0.47, MeOH). IR ν_{max} (film) cm^{-1}: 3395, 29668, 2930, 2874, 1648, 1580, 1511, 1454, 1370, 1262, 1159, 1075, 1041; ^{1}H-NMR (CD_3OD) δ: 0.95 (3H, s, H_3-12), 1.08 (3H, s, H_3-11), 1.19 (1H, ddd, J=13, 3, 1 Hz, H-2eq), 1.28 (3H, d, J=6 Hz, H_3-10), 1.34 (3H, s, H_3-13), 1.66 (1H, dd, J=13, 13 Hz, H-2ax), 3.65 (1H, dd, J=12, 6 Hz, H-6′a), 3.79 (1H, ddd, J=12, 3, 3 Hz, H-3), 3.86 (1H, dd, J=12, 2 Hz, H-6′b), 3.88 (1H, dd, J=3, 1 Hz, H-4), 4.28 (1H, d, J=8 Hz, H-1′), 4.52 (1H, dqd, J=7, 6, 1 Hz, H-9), 5.58 (1H, dd, J=16, 7 Hz, H-8), 6.04 (1H, dd, J=16, 1 Hz, H-7); ^{13}C-NMR (CD_3OD): Table 1; HR-FAB-MS (negative-ion mode) m/z: 403.1993 [M−H]$^-$ (Calcd for $C_{19}H_{31}O_9$: 403.1968).

Staphylionoside D (**6**): Amorphous powder, $[\alpha]_D^{26}$ −60.8° (c=0.21, MeOH). IR ν_{max} (film) cm^{-1}: 3395, 2965, 2929, 2878, 1938, 1667, 1608, 1243, 1075, 1020; UV λ_{max} (MeOH) nm (log ε): 233 (4.01); ^{1}H-NMR (CD_3OD) δ: 1.16 (3H, s, H_3-12), 1.38 (3H, s, H_3-11), 1.39 (3H, s, H_3-13), 1.46 (1H, dd, J=13, 13 Hz, H-4ax), 1.47 (1H, dd, J=13, 13 Hz, H-2ax), 2.10 (1H, ddd, J=13, 4, 2 Hz, H-2eq), 2.19 (3H, s, H_3-10), 2.38 (1H, ddd, J=13, 4, 2 Hz, H-4eq), 3.16 (1H, dd, J=9, 8 Hz, H-2′), 3.69 (1H, dd, J=12, 5 Hz, H-6′a), 3.88 (1H, dd, J=12, 2 Hz, H-6′b), 4.35 (1H, dddd, J=13, 13, 4, 4 Hz, H-3), 4.46 (1H, d, J=8 Hz, H-1′), 5.83 (1H, s, H-8); ^{13}C-NMR (CD_3OD): Table 1; HR-FAB-MS (negative-ion mode) m/z: 385.1875 [M−H]$^-$ (Calcd for $C_{19}H_{29}O_8$: 385.1862).

Staphylionoside E (**7**): Amorphous powder, $[\alpha]_D^{23}$ −99.0° (c=0.67, MeOH). IR ν_{max} (film) cm^{-1}: 3367, 2964, 2928, 2869, 1649, 1453, 1417, 1368, 1278, 1228, 1076, 1055, 1037; UV λ_{max} (MeOH) nm (log ε): 226 (3.74); ^{1}H-NMR (CD_3OD) δ: 1.05 (3H, s, H_3-12), 1.07 (3H, s, H_3-11), 1.27 (3H, d, J=7 Hz, H_3-10), 1.57 (1H, ddd, J=13, 4, 1 Hz, H-4eq), 1.85 (3H, s, H_3-13), 1.89 (1H, dd, J=13, 13 Hz, H-2ax), 3.67 (1H, dd, J=12, 5 Hz, H-6′a), 3.86 (1H, dd, J=12, 2 Hz, H-6′b), 3.98 (1H, ddd, J=13, 4, 4 Hz, H-3), 4.08 (1H, br d, J=4 Hz, H-4), 4.30 (1H, qd, J=6, 6 Hz, H-9), 4.50 (1H, d, J=8 Hz, H-1′), 5.53 (1H, dd, J=16, 6 Hz, H-8), 6.04 (1H, d, J=16 Hz, H-7); ^{13}C-NMR (CD_3OD): Table 1; HR-FAB-MS (negative-ion mode) m/z: 387.2007 [M−H]$^-$ (Calcd for $C_{19}H_{31}O_8$: 387.2019).

Staphylionoside F (**8**): Amorphous powder, $[\alpha]_D^{26}$ −118.1° (c=0.53, MeOH). IR ν_{max} (film) cm^{-1}: 3367, 2963, 2928, 2869, 1648, 1452, 1368, 1311, 1266, 1158, 1101, 1058, 1034; UV λ_{max} (MeOH) nm (log ε): 281 (2.90) sh, 226 (3.73); ^{1}H-NMR (CD_3OD) δ: 1.07 (3H, s, H_3-12), 1.08 (3H, s, H_3-11), 1.32 (3H, d, J=7 Hz, H_3-10), 1.45 (1H, ddd, J=13, 4, 1 Hz, H-2eq), 1.78 (1H, dd, J=13, 13 Hz, H-2ax), 1.86 (3H, s, H_3-13), 3.66 (1H, dd, J=12, 6 Hz, H-6′a), 3.76 (1H, ddd, J=13, 4, 4 Hz, H-3), 3.84 (1H, br d, J=4 Hz, H-3), 3.87 (1H, dd. J=12, 2 Hz, H-6′b), 4.39 (1H, d, J=8 Hz, H-1′), 4.52 (1H, qd, J=7, 7 Hz, H-9), 5.42 (1H, dd, J=16, 7 Hz, H-8), 6.14 (1H, d, J=16 Hz, H-7); ^{13}C-NMR (CD_3OD): Table 1; HR-FAB-MS (negative-ion mode) m/z: 387.2030 [M−H]$^-$ (Calcd for $C_{19}H_{31}O_8$: 387.2019).

Staphylionoside G (**9**): Amorphous powder, $[\alpha]_D^{26}$ −99.4° (c=1.21, MeOH). IR ν_{max} (film) cm^{-1}: 3395, 2962, 2926, 2871, 1648, 1452, 1368, 1313, 1262, 1158, 1076, 1033; UV λ_{max} (MeOH). (log ε): 233 (3.71); ^{1}H-NMR (CD_3OD) δ: 1.05 (3H, s, H_3-11), 1.07 (3H, s, H_3-12), 1.31 (3H, s, H_3-13), 1.52 (1H, dd, J=12, 12 Hz, H-4ax), 1.74 (3H, s, H3-13), 1.89 (1H, ddd, J=12, 4, 2 Hz, H-2eq), 2.08 (1H, dd, J=16, 12 Hz, H-4ax), 2.45 (1H, ddd, J=16, 6, 2 Hz, H-4eq), 3.67 (1H, dd, J=12, 6 Hz, H-6′a), 368 (1H, dd, J=12, 5 Hz, H-6″a), 3.86 (1H, dd, J=12, 2 Hz, H-6′b), 3.87 (1H, dd, J=12, 2 Hz, H-6″b), 4.10 (1H, dddd, J=12, 12, 6, 4 Hz, H-3), 4.40 (1H, d, J=8 Hz, H-1′), 4.43 (1H, d, J=8 Hz, H-1″), 4.50 (1H, dq, J=8, 7 Hz, H-9), 5.53 (1H, dd, J=16, 8 Hz, H-8), 6.10 (1H, d, J=16 Hz, H-7); ^{13}C-NMR (CD_3OD): Table 1; HR-FAB-MS (negative-ion mode) m/z: 533.2569 [M−H]$^-$ (Calcd for $C_{25}H_{41}O_{12}$: 533.2598).

Staphylionoside H (**10**): Amorphous powder, $[\alpha]_D^{26}$ −78.8° (c=3.40, MeOH). IR ν_{max} (film) cm^{-1}: 3395, 2966, 2928, 2875, 1648, 1592, 1452, 1368, 1311, 1268, 1158, 1076, 1036; ^{1}H-NMR (CD_3OD) δ: 0.95 (3H, s, H_3-12), 1.12 (3H, s, H_3-11), 1.22 (1H, dd, J=13, 10 Hz, H-2ax), 1.25 (3H, s, H_3-13), 1.28 (3H, d, J=6 Hz, H-10), 1.56 (1H, ddd, J=13, 5, 2 Hz, H-2eq), 1.62 (1H, dd, J=14, 9 Hz, H-4ax), 3.65 (1H, dd, J=12, 6 Hz, H-6′a), 3.75 (1H, m, H-3), 3.86 (1H, dd, J=12, 2 Hz, H-6′b), 4.28 (1H, d, J=8 Hz, H-1′), 4.52 (1H, qdd, J=6, 6, 1 Hz, H-9), 5.58 (1H, dd, J=16, 6 Hz, H-8), 6.05 (1H, dd, J=16, 1 Hz, H-7); ^{13}C-NMR (CD_3OD): Table 1; HR-FAB-MS (negative-ion mode) m/z 387.2008 [M−H]$^-$ (Calcd for $C_{19}H_{31}O_8$: 387.2019).

Staphylionoside I (**11**): Amorphous powder, $[\alpha]_D^{28}$ −34.4° (c=0.41, MeOH). IR ν_{max} (film) cm^{-1}: 3395, 2966, 1650, 1454, 1370, 1160, 1077,

1033; ^{1}H-NMR (CD_3OD) δ: 0.96 (3H, s, H_3-11), 1.15 (3H, s, H_3-12), 1.25 (3H, s, H_3-13), 1.34 (3H, d, J=7 Hz, H_3-10), 1.39 (1H, ddd, J=13, 5, 2 Hz, H-2eq), 1.70 (1H, dd, J=12, 12 Hz, H-2ax), 1.71 (1H, ddd, J=12, 5, 2 Hz, H-4eq), 1.76 (1H, dd, J=12, 12 Hz, H-4ax), 3.36 (3H, s, –OCH_3), 3.66 (1H, dd, J=12, 6 Hz, H-6′a), 3.86 (1H, dd, J=12, 2 Hz, H-6′b), 4.02 (1H, dddd, J=12, 12, 5, 5 Hz, H-3), 4.42 (1H, d, J=8 Hz, H-1′), 4.45 (1H, qd, J=7, 7 Hz, H-9), 5.83 (1H, dd, J=17, 7 Hz, H-8), 5.91 (1H, d, J=17 Hz, H-7); ^{13}C-NMR (CD_3OD): Table 1; HR-FAB-MS (negative-ion mode) m/z: 419.2265 $[M-H]^-$ (Calcd for $C_{20}H_{35}O_9$: 419.2281).

Staphylionoside J (**12**): Amorphous powder, $[\alpha]_D^{28}$ −37.6° (c=1.46, MeOH). IR ν_{max} (film) cm^{-1}: 3395, 2964, 2929, 1646, 1620, 1512, 1452, 1370, 1267, 1162, 1075, 1033; ^{1}H-NMR (CD_3OD) δ: 0.83 (3H, s, H_3-12), 1.03 (3H, s, H_3-11), 1.20 (3H, s, H_3-13), 1.29 (3H, d, J=6 Hz, H_3-10), 1.37 (1H, dd, J=13, 4 Hz, H-2eq), 1.64 (1H, dd, J=13, 13 Hz, H-2ax), 1.89 (1H, d, J=10 Hz, H-6), 3.49 (1H, d, J=3 Hz, H-4eq), 3.67 (1H, dd, J=12, 6 Hz, H-6′a), 3.88 (1H, dd, J=12, 2 Hz, H-6′b), 4.14 (1H, ddd, J=13, 4, 3 Hz, H-3ax), 4.42 (1H, d, J=8 Hz, H-1′), 4.47 (1H, dq, J=8, 6 Hz, H-9), 5.38 (1H, dd, J=16, 8 Hz, H-8), 5.80 (1H, dd, J=16, 10 Hz, H-7); ^{13}C-NMR (CD_3OD): Table 1; HR-FAB-MS (negative-ion mode) m/z: 405.2140 $[M-H]^-$ (Calcd for $C_{19}H_{33}O_9$: 405.2125).

Staphylionoside K (**13**): Amorphous powder, $[\alpha]_D^{28}$ −67.5° (c=0.53, MeOH). IR ν_{max} (film) cm^{-1}: 3395, 2927, 2877, 1648, 1454, 1376, 1313, 1262, 1159, 1077, 1024; ^{1}H-NMR (CD_3OD) δ: 1.05 (3H, s, H_3-11), 1.08 (3H, s, H_3-12), 1.26 (3H, d, J=6 Hz, H_3-10), 1.47 (1H, dd, J=12, 12 Hz, H-2ax), 1.53 (2H, m, H_2-8), 1.65 (3H, s, H_3-13), 1.83 (1H, ddd, J=12, 4, 2 Hz, H-2eq), 2.01 (1H, br dd, J=17, 10 Hz, H-4ax), 2.10 (2H, m, H_2-7), 2.33 (1H, br dd, J=17, 4 Hz, H-4eq), 3.64 (1H, dd, J=12, 6 Hz, H-6′a), 3.67 (1H, dd, J=12, 5 Hz, H-6″a), 3.83 (1H, dd, J=12, 2 Hz, H-6′a), 3.84 (1H, m, H-9), 3.85 (1H, dd, J=12, 2 Hz, H-6″b), 4.14 (1H, ddd, J=13, 4, 3 Hz, H-3ax), 4.34 (1H, d, J=8 Hz, H-1′), 4.44 (1H, d, J=8 Hz, H-1″); ^{13}C-NMR (CD_3OD): Table 1; HR-FAB-MS (negative-ion mode) m/z: 535.2783 $[M-H]^-$ (Calcd for $C_{25}H_{43}O_{12}$: 535.2755).

Enzymatic Hydrolysis of Staphylionoside A (3) Staphylionoside A (**3**) (6.1 mg) in 2 ml of H_2O was hydrolyzed with emulsin (5 mg) for 15 h at 37 °C. The reaction mixture was evaporated to dryness, and the methanolic solution was absorbed on silica gel and then subjected to silica gel CC (20 g, Φ=15 mm, L=20 cm) with $CHCl_3$ (100 ml) and $CHCl_3$–MeOH (19 : 1, 100 ml, 9 : 1, 100 ml, 17 : 3, 100 ml and 7 : 3, 300 ml), 10 ml fractions being collected. The aglycone (3.1 mg, 88%) and D-glucose (2.3 mg, 82%) were recovered in fractions 23—28 and 40—48, respectively. Aglycone (**3a**): Colorless syrup. $[\alpha]_D^{26}$ −91.9° (c=0.21, MeOH). ^{1}H-NMR (CD_3OD) δ: 1.16 (3H, s, H_3-11), 1.29 (3H, s, H_3-12), 1.30 (3H, d, J=7 Hz, H_3-10), 1.80 (1H, dd, J=14, 13 Hz, H-2ax), 1.83 (3H, s, H_3-13), 2.06 (1H, dd, J=14, 6 Hz, H-2eq), 4.32 (1H, dd, J=13, 6 Hz, H-3), 4.38 (1H, qdd, J=7, 6, 1 Hz, H-9), 5.75 (1H, dd, J=16, 6 Hz, H-8), 6.39 (1H, dd, J=16, 1 Hz, H-7); ^{13}C-NMR (CD_3OD) δ: 13.7 (C-13), 23.6 (C-10), 26.0 (C-11), 30.8 (C-12), 37.8 (C-1), 47.0 (C-2), 69.0 (C-9), 70.5 (C-3), 125.5 (C-7), 128.9 (C-5), 143.1 (C-8), 162.8 (C-6), 201.9 (C-4); HR-FAB-MS (negative-ion mode) m/z: 223.1315 $[M-H]^-$ (Calcd for $C_{13}H_{19}O_3$: 223.1334). D-Glucose, $[\alpha]_D^{22}$ +39.8° (c=0.15, H_2O, 24 h after being dissolved in the solvent).

Preparation of (*R*)- and (*S*)-MPTA Esters (3b, c) from the Aglycone of 3 (3a) A solution of **3a** (1.6 mg) in 1 ml of dehydrated CH_2Cl_2 was reacted with (*R*)-MPTA (30 mg) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)cardodiimide hydrochloride (EDC) (29 mg) and 4-*N*,*N*-dimethylaminopyridine (4-DMAP) (10 mg), and then the mixture was occasionally stirred at 25 °C for 30 min. After the addition of 1 ml of CH_2Cl_2, the solution was washed with H_2O (1 ml), 5% HCl (1 ml), $NaHCO_3$-saturated H_2O, and then brine (1 ml), successively. The organic layer was dried over Na_2SO_4 and then evaporated under reduced pressure. The residue was purified by preparative TLC [silica gel (0.25 mm thickness), applied for 18 cm and developed with $CHCl_3$–$(CH_3)_2CO$ (9 : 1) for 9 cm and eluted with $CHCl_3$–MeOH (9 : 1)] to furnish the ester, **3b** (2.2 mg, 45 %). Through a similar procedure, **3c** (1.3 mg, 27 %) was prepared from **3a** (1.6 mg) using (*S*)-MTPA (28 mg), EDC (29 mg), and 4-DMAP (11 mg).

(3*S*,9*S*,5*Z*,9*E*)-Megastigma-5,7-diene-3,9-dihydroxy-4-one 3,9-Di-*O*-(*R*)-MTPA Ester (**3b**): Amorphous powder; ^{1}H-NMR ($CDCl_3$) δ: 1.10 (3H, s, H_3-12), 1.28 (3H, s, H_3-11), 1.49 (3H, d, J=7 Hz, H_3-10), 1.77 (3H, d, J=1 Hz, H_3-13), 2.07 (1H, dd, J=13, 6 Hz, H-2eq), 2.21 (1H, dd, J=14, 13 Hz, H-2ax), 3.48 (3H, q, J=1 Hz, –OCH_3), 3.60 (3H, q, J=1 Hz, –OCH_3), 5.60 (1H, br dd, J=16, 7 Hz, H-8), 5.66 (1H, dd, J=14, 6 Hz, H-3), 5.68 (1H, br qd, J=7, 7 Hz, H-9), 6.21 (1H, br d, J=16 Hz, H-7), 7.36—7.44 (6H, m), 7.52—7.53 (2H, m), 7.69—7.71 (2H, m) (aromatic protons); HR-FAB-MS (positive-ion mode, *m*-nitrobenzyl alcohol as a matrix) m/z: 679.2111 $[M+Na]^+$ (+NaI) (Calcd for $C_{33}H_{34}O_7F_6Na$: 679.2106).

(3*S*,9*S*,5*Z*,7*E*)-Megastigma-5,7-diene-3,9-dihydroxy-4-one 3,9-Di-*O*-(*S*)-MTPA Ester (**3c**): Amorphous powder; ^{1}H-NMR ($CDCl_3$) δ: 1.09 (3H, s, H_3-12), 1.31 (3H, s, H_3-11), 1.46 (3H, d, J=7 Hz, H_3-10), 1.81 (3H, d, J=1 Hz, H_3-13), 1.96 (1H, dd, J=13, 6 Hz, H-2eq), 2.08 (1H, dd, J=14, 13 Hz, H-2ax), 3.52 (3H, q, J=1 Hz, –OCH_3), 3.69 (3H, q, J=1 Hz, –OCH_3), 5.60 (1H, br dd, J=16, 7 Hz, H-8), 5.66 (1H, br qd, J=7, 7 Hz, H-9), 5.70 (1H, dd, J=14, 6 Hz, H-3), 6.31 (1H, br d, J=16 Hz, H-7), 7.38—7.43 (6H, m), 7.52—7.53 (2H, m), 7.69—7.73 (2H, m) (aromatic protons); HR-FAB-MS (positive-ion mode, *m*-nitrobenzyl alcohol as a matrix) m/z: 679.2110 $[M+Na]^+$ (+NaI) (Calcd for $C_{33}H_{34}O_7F_6Na$: 679.2106).

Enzymatic Hydrolysis of Staphylionoside B (4) Staphylionoside B (12 mg) was hydrolyzed with emulsin (9 mg) for 21 h at 37 °C. A similar chromatographic workup to in the case of **3** gave 5.2 mg (68%) of an aglycone (**4a**) and 4.7 mg (84%) of D-glucose. Aglycone (**4a**): Colorless syrup. ^{1}H-NMR (CD_3OD) δ: 0.90 (3H, s, H_3-11), 1.16 (3H, s, H_3-13), 1.18 (3H, s, H_3-12), 1.27 (3H, d, J=6 Hz, H_3-10), 1.57 (1H, ddd, J=14, 5, 2 Hz, H-2eq), 1.71 (1H, ddd, J=14, 5, 2 Hz, H-4eq), 1.80 (1H, dd, J=14, 4 Hz, H-2ax), 2.04 (1H, dd, J=14, 4 Hz, H-4ax), 4.08 (1H, m, H-3), 4.34 (1H, qdd, J=6, 6, 1 Hz, H-9), 5.77 (1H, dd, J=16, 6 Hz, H-8), 6.15 (1H, dd, J=16, 1 Hz, H-7); ^{13}C-NMR (CD_3OD) δ: 24.1 (C-10), 26.1 (C-13), 27.7 (C-11), 29.0 (C-12), 38.8 (C-1), 44.2 (C-2), 69.0 (C-3), 69.6 (C-9), 77.6 (C-5), 80.2 (C-6), 131.9 (C-7), 135.5 (C-8); HR-FAB-MS (negative-ion mode) m/z: 243.1561 $[M-H]^-$ (Calcd for $C_{13}H_{23}O_4$: 243.1596). D-Glucose, $[\alpha]_D^{24}$ +32.3° (c=0.15, H_2O, 24 h after being dissolved in the solvent).

Preparation of (*R*)- and (*S*)-MPTA Esters (4b, c) from 4a Using a similar manner to as for the preparation of **3b** and **3c** from **3a**, **4b** and **4c** were prepared from **4a** (1.3 mg each) with the respective amounts of the reagents, (*R*)- and (*S*)-MPTA (46, 38 mg), EDC (36, 27 mg), and DMAP (44, 41 mg). The usual workup gave 3.0 mg (**4b**, 76%) and 3.0 mg (**4c**, 76%) of diesters, respectively.

(3*R*,5*R*,6*R*,9*S*,7*E*)-Megastigman-7-ene-3,5,6,9-tetrol 3,9-Di-*O*-(*R*)-MTPA Ester (**4b**): Amorphous powder; ^{1}H-NMR ($CDCl_3$) δ: 0.86 (3H, s, H_3-11), 0.98 (3H, s, H_3-13), 1.05 (3H, s, H_3-12), 1.44 (3H, d, J=6 Hz, H_3-10), 1.63 (1H, ddd, J=14, 5, 2 Hz, H-2eq), 1.70 (1H, ddd, J=14, 5, 2 Hz, H-4eq), 1.94 (1H, dd, J=14, 4 Hz, H-2ax), 2.16 (1H, dd, J=14, 4 Hz, H-4ax), 3.52 (3H, s, –OCH_3), 3.54 (3H, s, –OCH_3), 5.43 (1H, m, H-3), 5.65 (1H, m, H-9), 5.66 (1H, dd, J=16, 6 Hz, H-8), 6.29 (1H, d, J=16 Hz, H-7), 7.25—7.53 (10H, m, aromatic protons); HR-FAB-MS (positive-ion mode, *m*-nitrobenzyl alcohol as a matrix) m/z: 699.2367 $[M+Na]^+$ (+NaI) (Calcd for $C_{33}H_{38}O_8F_6Na$: 699.2369).

(3*R*,5*R*,6*R*,9*S*,7*E*)-Megastigman-7-ene-3,5,6,9-tetrol 3,9-Di-*O*-(*S*)-MTPA Ester (**4c**): Amorphous powder; ^{1}H-NMR ($CDCl_3$) δ: 0.76 (3H, s, H_3-12), 0.82 (3H, s, H_3-11), 1.10 (3H, s, H_3-13), 1.39 (3H, d, J=6 Hz, H_3-10), 1.56 (1H, ddd, J=14, 5, 2 Hz, H-2eq), 1.83 (1H, ddd, J=14, 5, 2 Hz, H-4eq), 1.89 (1H, dd, J=14, 4 Hz, H-2ax), 2.22 (1H, dd, J=14, 4 Hz, H-4ax), 3.51 (3H, s, –OCH_3), 3.58 (3H, s, –OCH_3), 5.44 (1H, m, H-3), 5.62 (1H, dq, J=6, 6 Hz, H-9), 5.75 (1H, dd, J=16, 6 Hz, H-8), 6.35 (1H, d, J=16 Hz, H-7), 7.26—7.56 (10H, m, aromatic protons); HR-FAB-MS (positive-ion mode, *m*-nitrobenzyl alcohol as a matrix) m/z: 699.2332 $[M+Na]^+$ (+NaI) (Calcd for $C_{33}H_{38}O_8F_6Na$: 699.2369).

Enzymatic Hydrolysis of Staphylionoside C (5) Staphylionoside C (7 mg) was hydrolyzed with emulsin (8 mg) for 21 h at 37 °C. A similar chromatographic workup to in the case of **3** gave 3.4 mg (70%) of an aglycone (**5a**) and 2.8 mg (89%) of D-glucose. Aglycone (**5a**): Colorless syrup. ^{1}H-NMR (CD_3OD) δ: 0.95 (3H, s, H_3-11), 1.08 (3H, s, H_3-12), 1.20 (1H, ddd, J=13, 4, 1 Hz, H-2eq), 1.23 (3H, d, J= 6 Hz, H_3-10), 1.30 (3H, s, H_3-13), 1.65 (1H, dd, J=13, 13 Hz, H-2ax), 3.79 (1H, ddd, J=13, 4, 4 Hz, H-3), 3.88 (1H, dd, J=4, 1 Hz, H-4), 4.29 (1H, qdd, J=6, 5, 1 Hz, H-9), 5.66 (1H, dd, J=16, 5 Hz, H-8), 5.90 (1H, dd, J=16, 1 Hz, H-7); ^{13}C-NMR (CD_3OD) δ: 17.3 (C-13), 23.9 (C-10), 24.9 (C-12), 29.7 (C-11), 35.3 (C-1), 40.6 (C-2), 66.7 (C-3), 68.7 (C-9), 69.8 (C-5), 71.7 (C-6), 73.3 (C-4), 125.9 (C-7), 139.3 (C-8); HR-FAB-MS (negative-ion mode) m/z: 241.1436 $[M-H]^-$ (Calcd for $C_{13}H_{21}O_4$: 241.1440). D-Glucose, $[\alpha]_D^{23}$ +39.4° (c= 0.19, H_2O, 24 h after being dissolved in the solvent).

Preparation of (*R*)- and (*S*)-MPTA Esters (5b, c) from 5a Using a similar manner to as for the preparation of **3b** and **3c** from **3a**, **5b** and **5c** were prepared from **5a** (1.7 mg each) with the respective amounts of the reagents, (*R*)- and (*S*)-MPTA (40, 45 mg), EDC (26, 25 mg), and DMAP (33, 32 mg). The usual workup gave 1.3 mg (**5b**, 27%) and 2.0 mg (**5c**, 42%) of diesters, respectively.

(3*S*,4*S*,5*R*,6*S*,9*S*,7*E*)-5,6-Epoxymegastigman-7-ene-3,4,9-triol 3,9-Di-*O*-(*R*)-MTPA Ester (**5b**): Amorphous powder; ^{1}H-NMR ($CDCl_3$) δ: 1.01 (3H, s, H_3-12), 1.02 (3H, s, H_3-11), 1.21 (3H, s, H_3-13), 1.40 (1H, dd, J=13, 4 Hz, H-2eq), 1.43 (3H, d, J=6 Hz, H_3-11), 1.86 (1H, dd, J=13, 13 Hz, H-

2ax), 3.49 (3H, s, –OCH_3), 3.58 (3H, s, –OCH_3), 4.09 (1H, br d, J=4 Hz, H-4), 5.26 (1H, ddd, J=13, 4, 4 Hz, H-3), 5.62 (1H, qd, J=6, 6 Hz, H-9), 5.67 (1H, dd, J=15, 6 Hz, H-8), 5.94 (1H, d, J=15 Hz, H-7), 7.25—7.52 (10H, m, aromatic protons); HR-FAB-MS (positive-ion mode, *m*-nitrobenzyl alcohol as a matrix) *m/z*: 697.2149 $[M+Na]^+$ (+NaI) (Calcd for $C_{33}H_{36}O_8F_6Na$: 697.2212).

(3*S*,4*S*,5*R*,6*S*,9*S*,7*E*)-5,6-Epoxymegastigman-7-ene-3,4,9-triol 3,9-Di-*O*-(*S*)-MTPA Ester (**5c**): Amorphous powder; ^{1}H-NMR ($CDCl_3$) δ: 1.03 (3H, s, H_3-12), 1.05 (3H, s, H_3-11), 1.26 (3H, s, H_3-13), 1.38 (3H, d, J=6 Hz, H_3-10), 1.41 (1H, dd, J=13, 4 Hz, H-2eq), 1.78 (1H, dd, J=13, 13 Hz, H-2ax), 3.48 (3H, s, –OCH_3), 3.52 (3H, s, –OCH_3), 4.18 (1H, br d, J=4 Hz, H-4), 5.26 (1H, ddd, J=13, 4, 4 Hz, H-3), 5.61 (1H, qd, J=6, 6 Hz, H-9), 5.75 (1H, dd, J=15, 6 Hz, H-8), 6.02 (1H, d, J=15 Hz, H-7), 7.25—7.52 (10H, m, aromatic protons); HR-FAB-MS (positive-ion mode, *m*-nitrobenzyl alcohol as a matrix) *m/z*: 697.2206 $[M+Na]^+$ (+NaI) (Calcd for $C_{33}H_{36}O_8F_6Na$: 697.2212).

Enzymatic Hydrolysis of Staphylionoside E (7) Staphylionoside E (10 mg) was hydrolyzed with emulsin (18 mg) for 28 h at 37 °C. A similar chromatographic workup to in the case of **3** gave 5.0 mg (86%) of an aglycone (**7a**) and 4.0 mg (87%) of D-glucose. Aglycone (**7a**): Colorless syrup. $[\alpha]_D^{26}$ −101.0° (c=0.33, MeOH). ^{1}H-NMR (CD_3OD) δ: 1.04 (3H, s, H_3-11), 1.06 (3H, s, H_3-12), 1.27 (3H, d, J=6 Hz, H_3-10), 1.44 (1H, ddd, J=13, 4, 1 Hz, H-2eq), 1.78 (1H, dd, J=13, 13 Hz, H-2ax), 1.83 (3H, d, J=1 Hz, H_3-13), 3.75 (1H, ddd, J=13, 4, 4 Hz, H-3), 3.83 (1H, br d, J=4 Hz, H-4), 4.30 (1H, qdd, J=6, 6, 1 Hz, H-9), 5.52 (1H, dd, J=16, 6 Hz, H-8), 6.04 (1H, dd, J=16, 1 Hz, H-7); ^{13}C-NMR (CD_3OD) δ: 19.9 (C-13), 23.9 (C-10), 27.8 (C-11), 30.4 (C-12), 37.8 (C-1), 41.8 (C-2), 68.0 (C-3), 69.5 (C-9), 72.7 (C-4), 126.8 (C-7), 129.0 (C-5), 140.6 (C-8), 142.6 (C-6); HR-FAB-MS (negative-ion mode) *m/z*: 225.1492 $[M-H]^-$ (Calcd for $C_{13}H_{21}O_3$: 225.1491). D-Glucose, $[\alpha]_D^{23}$ +37.5° (c=0.26, H_2O, 24 h after being dissolved in the solvent).

Preparation of (*R*)- and (*S*)-MPTA Esters (7b, c) from 7a Using a similar manner to as for the preparation of **3b** and **3c** from **3a**, **7b** and **7c** were prepared from **7a** (1.6 mg each) with the respective amounts of the reagents, (*R*)- and (*S*)-MPTA (27, 29 mg), EDC (18, 19 mg), and DMAP (20, 26 mg). The usual workup gave 1.0 mg (**7b**, 21%) and 1.1 mg (**7c**, 24%) of diesters, respectively.

(3*S*,4*R*,9*S*,5*Z*,7*E*)-Megastigma-5,7-diene-3,4,9-triol 3,9-Di-*O*-(*R*)-MTPA Ester (**7b**): Amorphous powder; ^{1}H-NMR ($CDCl_3$) δ: 1.00 (3H, s, H_3-11), 1.01 (3H, s, H_3-12), 1.47 (3H, d, J=7 Hz, H_3-10), 1.63 (1H, ddd, J=13, 4, 1 Hz, H-2eq), 1.75 (3H, d, J=1 Hz, H_3-13), 2.03 (1H, dd, J=13, 13 Hz, H-2ax), 3.56 (3H, s, –OCH_3), 3.58 (3H, s, –OCH_3), 4.05 (1H, d, J=4 Hz, H-4), 5.21 (1H, ddd, J=13, 4, 4 Hz, H-3), 5.46 (1H, dd, J=16, 7 Hz, H-8), 5.64 (1H, qd, J=7, 7 Hz, H-9), 6.10 (1H, d, J=16 Hz, H-7), 7.26—7.55 (10H, m, aromatic protons); HR-FAB-MS (positive-ion mode, *m*-nitrobenzyl alcohol as a matrix) *m/z*: 681.2223 $[M+Na]^+$ (+NaI) (Calcd for $C_{33}H_{36}O_7F_6$: 681.2263).

(3*S*,4*R*,9*S*,5*Z*,7*E*)-Megastigma-5,7-diene-3,4,9-triol 3,9-Di-*O*-(*S*)-MTPA Ester (**7c**): Amorphous powder; ^{1}H-NMR ($CDCl_3$) δ: 1.00 (3H, s, H_3-11), 1.12 (3H, s, H_3-12), 1.41 (3H, d, J=7 Hz, H_3-10), 1.60 (1H, ddd, J=13, 4, 1 Hz, H-2eq), 1.81 (3H, d, J=1 Hz, H_3-13), 1.95 (1H, dd, J=13, 13 Hz, H-2ax), 3.53 (3H, s, –OCH_3), 3.56 (3H, s, –OCH_3), 4.16 (1H, br d, J=4 Hz, H-4), 5.20 (1H, ddd, J=13, 4, 4 Hz, H-3), 5.54 (1H, dd, J=16, 7 Hz, H-8), 5.63 (1H, qd, J=6, 6 Hz, H-9), 6.19 (1H, d, J=16 Hz, H-7), 7.25—7.55 (10H, m, aromatic protons); HR-FAB-MS (positive-ion mode, *m*-nitrobenzyl alcohol as a matrix) *m/z*: 681.2273 $[M+Na]^+$ (+NaI) (Calcd for $C_{33}H_{36}O_7F_6Na$: 681.2263).

Enzymatic Hydrolysis of Staphylionoside F (8) Staphylionoside F (10 mg) was hydrolyzed with emulsin (18 mg) for 18 h at 37 °C. A similar chromatographic workup to in the case of **3** gave 4.9 mg (76%) of an aglycone (**8a**) and 3.7 mg (73%) of D-glucose. Aglycone (**8a**): ^{1}H- and ^{13}C-NMR (CD_3OD): essentially the same as those of **7a**; HR-FAB-MS (negative-ion mode) *m/z*: 225.1495 $[M-H]^-$ (Calcd for $C_{13}H_{21}O_3$: 225.1491). D-Glucose, $[\alpha]_D^{23}$ +24.3° (c=0.25, H_2O, 24 h after being dissolved in the solvent).

Preparation of (*R*)- and (*S*)-MPTA Esters (8b, c) from 8a Using a similar manner to as for the preparation of **3b** and **3c** from **3a**, **8b** and **8c** were prepared from **8a** (1.7 mg each) with the respective amounts of the reagents, (*R*)- and (*S*)-MPTA (39, 35 mg), EDC (27, 26 mg), and DMAP (30, 33 mg). The usual workup gave 1.8 mg (**8b**, 39%) and 1.4 mg (**8c**, 30%) of diesters, respectively. (3*S*,4*R*,9*S*,5*Z*,7*E*)-Megastigma-5,7-diene-3,4,9-triol 3,9-Di-*O*-(*R*)-MTPA ester (**8b**) and (3*S*,4*R*,9*S*,5*Z*,7*E*)-megastigma-5,7-diene-3,4,9-triol 3,9-Di-*O*-(*S*)-MTPA ester (**8c**): Amorphous powders, ^{1}H-NMR ($CDCl_3$): Essentially the same as those of **7b** and **7c**, respectively; HR-FAB-MS (positive-ion mode, *m*-nitrobenzyl alcohol as a matrix) *m/z*: 681.2223 and 681.2273, respectively, $[M+Na]^+$ (+NaI) (Calcd for $C_{33}H_{36}O_7F_6Na$: 681.2263).

Enzymatic Hydrolysis of Staphylionoside H (10) Staphylionoside H (18 mg) was hydrolyzed with emulsin (8 mg) for 21 h at 37 °C. A similar chromatographic workup to in the case of **3** gave 6.6 mg (79%) of an aglycone (**10a**) and 8.7 mg (83%) of D-glucose. Aglycone (**10a**): Colorless syrup. $[\alpha]_D^{23}$ −75.0° (c=0.58, MeOH). ^{1}H-NMR (CD_3OD) δ: 0.94 (3H, s, H_3-12), 1.11 (3H, s, H_3-11), 1.20 (1H, dd, J=13, 10 Hz, H-2ax), 1.21 (3H, s, H_3-13), 1.23 (3H, d, J=7 Hz, H_3-10), 1.55 (1H, ddd, J=13, 3, 2 Hz, H-2eq), 1.59 (1H, dd, J=14, 9 Hz, H-4ax), 2.27 (1H, ddd, J=14, 5, 2 Hz, H-4eq), 3.75 (1H, m, H-3), 3.83 (1H, br d, J=4 Hz, H-4), 4.30 (1H, qdd, J=7, 7, 1 Hz, H-9), 5.68 (1H, dd, J=16, 7 Hz, H-8), 5.91 (1H, dd, J=16, 1 Hz, H-7); ^{13}C-NMR (CD_3OD) δ: 20.2 (C-13), 23.9 (C-10), 25.1 (C-12), 30.1 (C-11), 35.9 (C-1), 41.7 (C-4), 48.0 (C-2), 64.6 (C-3), 68.1 (C-5), 68.7 (C-8), 71.2 (C-6), 125.8 (C-7), 139.2 (C-8); HR-FAB-MS (negative-ion mode) *m/z*: 225.1517 $[M-H]^-$ (Calcd for $C_{13}H_{21}O_3$: 225.1491). D-Glucose, $[\alpha]_D^{19}$ +31.0° (c=0.58, H_2O, 24 h after being dissolved in the solvent).

Preparation of (*R*)- and (*S*)-MPTA Esters (10b, c) from 10a Using a similar manner to as for the preparation of **3b** and **3c** from **3a**, **10b** and **10c** were prepared from **10a** (2.2 mg each) with the respective amounts of the reagents, (*R*)- and (*S*)-MPTA (38, 42 mg), EDC (28, 28 mg), and DMAP (27, 24 mg). The usual workup gave 1.3 mg (**10b**, 21%) and 2.0 mg (**10c**, 33%) of diesters, respectively.

(3*S*,5*R*,6*R*,9*S*,7*E*)-Megastigman-5,6-epoxy-7-ene-3,9-diol di-*O*-(*R*)-MTPA Ester (**10b**): Amorphous powder; ^{1}H-NMR ($CDCl_3$) δ: 0.95 (3H, s, H_3-11), 0.98 (3H, s, H_3-12), 1.06 (3H, s, H_3-13), 1.38 (1H, dd, J=13, 9 Hz, H-2ax), 1.41 (3H, d, J=6 Hz, H_3-10), 1.74 (1H, ddd, J=13, 4, 1 Hz, H-2eq), 2.38 (1H, ddd, J=15, 6, 1 Hz, H-4eq), 3.52 (3H, s, –OCH_3), 3.54 (3H, s, –OCH_3), 5.16 (1H, m, H-3), 5.61 (1H, dq, J=6, 6 Hz, H-9), 5.63 (1H, dd, J=14, 6 Hz, H-8), 5.89 (1H, d, J=14 Hz, H-7), 7.25—7.52 (10H, m, aromatic protons); HR-FAB-MS (negative-ion mode) *m/z*: 627.2198 $[M-OCH_3]^-$ (Calcd for $C_{32}H_{33}O_6F_6$: 627.2181).

(3*S*,5*R*,6*R*,9*S*,7*E*)-Megastigman-5,6-epoxy-7-ene-3,9-diol di-*O*-(*S*)-MTPA Ester (**10c**): Amorphous powder; ^{1}H-NMR ($CDCl_3$) δ: 0.96 (3H, s, H_3-11), 0.97 (3H, s, H_3-12), 1.14 (3H, s, H_3-13), 1.31 (1H, dd, J=13, 9 Hz, H-2ax), 1.37 (3H, d, J=6 Hz, H_3-10), 1.70 (1H, ddd, J=13, 4, 1 Hz, H-2eq), 1.86 (1H, dd, J=15, 7 Hz, H-4ax), 2.47 (1H, ddd, J=15, 6, 1 Hz, H-4eq), 3.51 (3H, s, –OCH_3), 3.53 (3H, s, –OCH_3), 5.17 (1H, m, H-3), 5.60 (1H, dq, J=6, 6 Hz, H-9), 5.75 (1H, dd, J=14, 6 Hz, H-8), 6.01 (1H, d, J=14 Hz, H-7), 7.26—7.52 (10H, m, aromatic protons); HR-FAB-MS (negative-ion mode *m/z*: 627.2156 $[M-OCH_3]^-$ (Calcd for $C_{32}H_{33}O_6F_6$: 627.2181).

Enzymatic Hydrolysis of Staphylionoside I (11) Staphylionoside I (6 mg) was hydrolyzed with emulsin (5 mg) for 30 h at 37 °C. A similar chromatographic workup to in the case of **3** gave 3.3 mg (89%) of an aglycone (**11a**) and 2.5 mg (97%) of D-glucose. Aglycone (**11a**): Colorless syrup. $[\alpha]_D^{26}$ +22.7° (c=0.22, MeOH). ^{1}H-NMR (CD_3OD) δ: 0.93 (3H, s, H_3-11), 1.13 (3H, s, H_3-12), 1.24 (3H, s, H_3-13), 1.29 (3H, d, J=6 Hz, H_3-10), 1.38 (1H, ddd, J=12, 4, 2 Hz, H-2eq), 1.69 (1H, dd, J=12, 12 Hz, H-2ax), 1.70 (1H, ddd, J=13, 5, 2 Hz, H-4eq), 1.76 (1H, dd, J=13, 11 Hz, H-4ax), 3.34 (3H, s, –OCH_3), 4.02 (1H, dddd, J=12, 11, 5, 4 Hz, H-3), 4.32 (1H, qd, J=6, 1 Hz, H-9), 5.80 (1H, dd, J=16, 1 Hz, H-7), 5.96 (1H, dd, J=16, 6 Hz, H-8); ^{13}C-NMR (CD_3OD) δ: 24.1 (C-10), 26.2 (C-12), 28.1 (C-11), 38.6 (C-13), 42.9 (C-1), 46.4 (C-2 or 4), 46.6 (C-4 or 2), 55.4 (–OCH_3), 65.4 (C-3), 70.1 (C-9), 78.5 (C-5), 84.5 (C-6), 125.5 (C-7), 140.8 (C-8); HR-FAB-MS (negative and positive-ion modes): no peak assignable to a quai-molecular ion was observed. D-Glucose, $[\alpha]_D^{19}$ +43.2° (c=0.17, H_2O, 24 h after being dissolved in the solvent).

Preparation of (*R*)- and (*S*)-MPTA Esters (11b, c) from 11a Uing a similar manner to as for the preparation of **3b** and **3c** from **3a**, **11b** and **11c** were prepared from **11a** (1.6 mg each) with the respective amounts of the reagents, (*R*)- and (*S*)-MPTA (36, 39 mg), EDC (23, 28 mg), and DMAP (34, 32 mg). The usual workup gave 2.0 mg (**11b**, 47%) and 3.7 mg (**11c**, 86%) of diesters, respectively.

(3*S*,5*R*,6*R*,9*S*,7*E*)-Megastigman-7-ene-6-methoxy-3,5,9-triol Di-*O*-(*R*)-MTPA Ester (**11b**): Amorphous powder; ^{1}H-NMR ($CDCl_3$) δ: 0.89 (3H, s, H_3-11), 1.14 (3H, s, H_3-12), 1.20 (3H, s, H_3-13), 1.49 (3H, d, J=6 Hz, H_3-10), 1.73 (1H, ddd, J=13, 5, 2 Hz, H-4eq), 1.92 (1H, dd, J=13, 12 Hz, H-2ax), 1.96 (1H, dd, J=13, 11 Hz, H-4ax), 3.27 (3H, s, –OCH_3 on C-6), 3.55 (3H, s, –OCH_3), 3.57 (3H, s, –OCH_3), 5.43 (1H, dddd, J=12, 12, 5, 5 Hz, H-3), 5.63 (1H, dq, J=6, 6 Hz, H-9), 5.88 (1H, d, J=16 Hz, H-7), 5.96 (1H, dd, J=16, 6 Hz, H-8), 7.25—7.53 (10H, m, aromatic protons); HR-FAB-MS (negative-ion mode) *m/z*: 713.2515 $[M+Na]^+$ (+NaI) (Calcd for $C_{34}H_{40}O_8F_6Na$: 713.2525).

(3*S*,5*R*,6*R*,9*S*,7*E*)-Megastigman-7-ene-6-methoxy-3,5,9-triol Di-*O*-(*S*)-MTPA Ester (**11c**): Amorphous powder; ^{1}H-NMR ($CDCl_3$) δ: 0.90 (3H, s,

H_3-11), 1.16 (3H, s, H_3-12), 1.26 (3H, s, H_3-13), 1.44 (3H, d, J=6 Hz, H_3-10), 1.83 (1H, ddd, J=13, 5, 2 Hz, H-4eq), 1.85 (1H, dd, J=12, 12 Hz, H-2ax), 1.85 (1H, dd, J=12, 12 Hz, H-2ax), 2.07 (1H, dd, J=12, 11 Hz, H-4ax), 3.30 (3H, s, $-OCH_3$ on C-6), 3.53 (3H, s, $-OCH_3$), 3.55 (3H, s, $-OCH_3$), 5.44 (1H, dddd, J=12, 12, 5, 5 Hz, H-3), 5.61 (1H, dq, J=6, 6 Hz, H-9), 5.96 (1H, d, J=16 Hz, H-7), 6.05 (1H, dd, J=16, 6 Hz, H-8), 7.26—7.60 (10H, m, aromatic protons); HR-FAB-MS (negative-ion mode *m*/*z*: 713.2547 $[M+Na]^+$ (+NaI) (Calcd for $C_{34}H_{40}O_8F_6Na$: 713.2525).

Enzymatic Hydrolysis of Staphylionoside J (12) Staphylionoside J (11 mg) was hydrolyzed with emulsin (5 mg) for 21 h at 37 °C. A similar chromatographic workup to in the case of **3** gave 4.0 mg (60%) of an aglycone (**12a**) and 4.3 mg (88%) of D-glucose. Aglycone (**11a**): Colorless syrup. ^{1}H-NMR (CD_3OD) δ: 0.92 (3H, s, H_3-12), 1.03 (3H, s, H_3-11), 1.16 (3H, s, H_3-13), 1.25 (3H, d, J=6 Hz, H_3-10), 1.37 (1H, ddd, J=13, 4, 1 Hz, H-2eq), 1.64 (1H, dd, J=13, 13 Hz, H-2ax), 1.82 (1H, d, J=10 Hz, H-6), 3.48 (1H, dd, J=3 Hz, H-4eq), 4.13 (1H, ddd, J=13, 4, 3 Hz, H-3), 4.26 (1H, qd, J=6, 1 Hz, H-9), 5.48 (1H, dd, J=16, 6 Hz, H-8), 5.72 (1H, ddd, J=16, 10, 1 Hz, H-7); ^{13}C-NMR (CD_3OD) δ: 23.6 (C-11), 24.1 (C-10), 28.0 (C-13), 33.1 (C-12), 35.5 (C-1), 43.4 (C-2), 53.2 (C-6), 67.0 (C-3), 69.6 (C-9), 76.3 (C-5), 78.5 (C-4), 128.2 (C-7), 139.9 (C-8); HR-FAB-MS (negative-ion mode) *m*/*z*: 243.1609 $[M-H]^-$ (Calcd for $C_{13}H_{23}O_4$: 243.1596). D-Glucose, $[\alpha]_D^{19}$ +42.9° (c=0.28, H_2O, 24 h after being dissolved in the solvent).

Preparation of (*R*)- and (*S*)-MPTA Esters (12b, c) from 12a Using a similar manner to as for the preparation of **3b** and **3c** from **3a**, **12b** and **12c** were prepared from **12a** (2.0 mg each) with the respective amounts of the reagents, (*R*)- and (*S*)-MPTA (38, 39 mg), EDC (28, 29 mg), and DMAP (39, 37 mg). The usual workup gave 2.5 mg (**12b**, 44%) and 2.2 mg (**12c**, 38%) of diesters, respectively.

(3*S*,4*R*,5*R*,6*R*,9*S*,7*E*)-Megastigman-7-ene-3,4,5,9-triol 3,9-Di-*O*-(*R*)-MTPA Ester (**12b**): Amorphous powder; ^{1}H-NMR ($CDCl_3$) δ: 0.81 (3H, s, H_3-12), 1.08 (3H, s, H_3-11), 1.15 (3H, s, H_3-13), 1.44 (3H, d, J=6 Hz, H_3-10), 1.58 (1H, ddd, J=12, 4, 1 Hz, H-2eq), 1.75 (1H, dd, J=12, 12 Hz, H-2ax), 1.89 (1H, d, J=10 Hz, H-6), 3.35 (3H, s, $-OCH_3$), 3.56 (3H, s, $-OCH_3$), 3.71 (1H, d, J=3 Hz, H-4), 5.45 (1H, dd, J=16, 6 Hz, H-8), 5.57 (1H, qd, J=6, 6 Hz, H-9), 5.62 (1H, ddd, J=12, 4, 3 Hz, H-3), 5.82 (1H, ddd, J=16, 10, 1 Hz, H-7), 7.25—7.55 (10H, m, aromatic protons); HR-FAB-MS (negative-ion mode) *m*/*z*: 699.2397 $[M+Na]^+$ (+NaI) (Calcd for $C_{33}H_{38}O_8F_6Na$: 699.2369).

(3*S*,4*R*,5*R*,6*R*,9*S*,7*E*)-Megastigman-7-ene-3,4,5,9-triol 3.9-Di-*O*-(*S*)-MTPA Ester (**12c**): Amorphous powder; ^{1}H-NMR ($CDCl_3$) δ: 0.87 (3H, s, H_3-12), 1.11 (3H, s, H_3-11), 1.14 (3H, s, H_3-13), 1.39 (3H, d, J=6 Hz, H_3-10), 1.61 (1H, ddd, J=12, 4, 2 Hz, H-2eq), 1.87 (1H, dd, J=12, 12 Hz, H-2ax), 1.93 (1H, d, J=10 Hz, H-6), 3.53 (3H, s, $-OCH_3$), 3.58 (3H, s, $-OCH_3$), 3.63 (1H, d, J=3 Hz, H-4), 5.63 (1H, ddd, J=12, 4, 3 Hz, H-3), 5.54 (1H, qd, J=16, 6 Hz, H-8), 5.59 (1H, dd, J=6, 6 Hz, H-9), 5.91 (1H, ddd, J=16, 10, 1 Hz, H-7), 7.26—7.54 (10H, m, aromatic protons); HR-FAB-MS (negative-ion mode *m*/*z*: 699.2369 $[M+Na]^+$ (+NaI) (Calcd for $C_{33}H_{38}O_8F_6Na$: 699.2369).

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