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Medicinal Foodstuffs. II.[1] On the Bioactive Constituents of the Tuber of *Sagittaria trifolia* L. (Kuwai, Alismataceae): Absolute Stereostructures of Trifoliones A, B, C, and D, Sagittariosides a and b, and Arabinothalictoside

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From the medicinal foodstuff "kuwai", the tuber of *Sagittaria trifolia* L., four bioactive diterpene ketones, trifoliones A, B, C, and D, two diterpene glucosides, sagittariosides a and b, and a nitroethylphenol glycoside, arabinothalictoside, were isolated, together with six known diterpenes. Their absolute stereostructures were determined on the basis of chemical and physicochemical evidence which included the application of a modified Mosher's method and an exciton chirality method. Among the diterpene constituents, trifoliones A, B, C, and D exhibited inhibitory effects on the histamine release from rat mast cells induced by compound 48/80 and calcium ionophore A-23187.

Key words medicinal foodstuff; *Sagittaria trifolia*; trifolione; sagittarioside; histamine release inhibitor; diterpene ketone

A Chinese traditional medicine " 慈姑 (zi gu)", the air-dried tuber of *Sagittaria trifolia* L. (*S. sagittifolia* L., Alismataceae), has been used medicinally during childbirth and for skin disease. On the other hand, the fresh tuber of this plant is called "kuwai" in Japanese and it has been used as a garnish foodstuff in Japanese-style dishes. In regard to the chemical constituents of *Sagittaria trifolia* L., a diterpene, isoabienol (**8**), has been isolated from the Japanese fresh tuber,[2] while another diterpene, sagittariol, was obtained from the whole plant collected in India.[3]

As a part of our characterization studies on antiallergic constituents of foodstuffs,[4] we have so far isolated four diterpene ketones, trifoliones A (**1**), B (**2**), C (**3**), and D (**4**), two diterpene glucosides, sagittariosides a (**5**) and b (**6**), and a phenolic glycoside containing a nitroethyl group, arabinothalictoside (**7**), together with six known diterpenes (**8**, **9**, **10**, **11**, **12**, **13**) from the fresh tuber of *Sagittaria trifolia* L. In this paper, we present a full account of the structure elucidation of these diterpenes (**1—4**) and glycosides (**5—7**) and we describe the inhibitory activities of the diterpene constituents on histamine release from rat mast cells.[5]

Fresh tubers of *Sagittaria trifolia* L. cultivated in Saitama Prefecture, Japan, were extracted with methanol, and the extract was partitioned into a mixture of ethyl acetate and water. The water-soluble portion was further extracted with 1-butanol. The ethyl acetate- and 1-butanol-soluble portions were subjected to separation and purification through the procedures shown in Chart
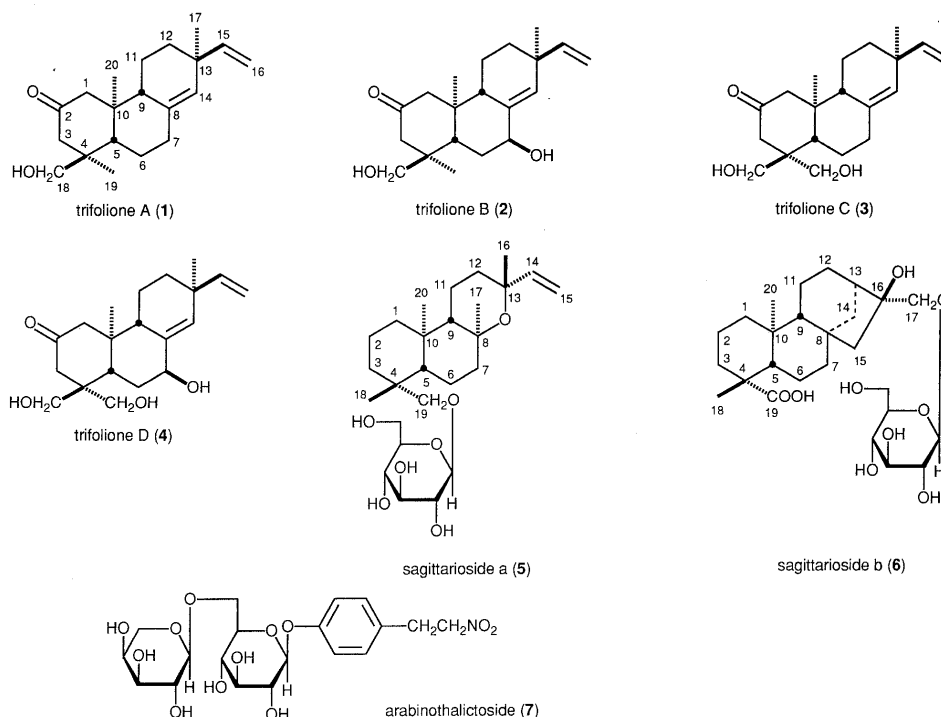

trifolione A (**1**)

trifolione B (**2**)

trifolione C (**3**)

trifolione D (**4**)

sagittarioside a (**5**)

sagittarioside b (**6**)

arabinothalictoside (**7**)

Chart 1

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isoabienol (8)

13-episclareol (9)

ent-13-epimanoyl oxide (10) : R=H
ent-19-hydroxy-13-epimanoyl oxide (11) :
R=OH

ent-kaur-16-en-19-ol (12) : R=CH₂OH
ent-kaur-16-en-19-oic acid (13) : R=COOH

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Chart 2

fresh tubers of *Sagittaria trifolia* L. (8 kg)

fr. 1 (lipid)

fr. 2 —— a, c) —— isoabienol (8, 0.003%)
ent-13-epimanoyloxide
(10, 0.0002%)

MeOH/Δ

MeOH ext. (7.5%)

AcOEt/H₂O

fr. 3 —— c) —— ent-kaur-16-en-19-ol
(12, 0.0001%)

H₂O layer   AcOEt layer (0.3%) —— a)

fr. 4 —— a, d) —— ent-kaur-16-en-19-oic acid
(13, 0.001%)
ent-19-hydroxy-13-epimanoyl oxide
(11, 0.003%)

n-BuOH

H₂O layer   n-BuOH layer (0.5%)

fr. 5 —— a) —— trifolione A (1, 0.0014%)

13-episclareol (9, 0.01%)

a)

fr. 1   fr. 2   fr. 3   fr. 4   fr. 5   fr. 6

a, e)   a, e)   a, b, d)   a, b)   a)   d)

daucosterin
(0.002%)

daucosterin
fatty acid ester
(0.004%)

trifolione B
(2, 0.0003%)
trifolione C
(3, 0.001%)
sagittarioside a
(5, 0.0003%)

trifolione D
(4, 0.0003%)

sagittarioside b
(6, 0.0001%)

arabinothalictoside
(7, 0.0006%)

a)   ordinary phase silica gel column chromatog.
b)   reversed phase silica gel column chromatog.
c)   recycled HPLC
d)   ODS HPLC
e)   Sephadex LH-20 column chromatog.

Chart 3

3. Namely, repeated separation of the ethyl acetate-soluble portion by ordinary and reversed-phase silica gel column chromatography and finally HPLC furnished trifolione A (1), isoabienol (8),[2] 13-episclareol (9),[6] ent-13-epimanoyl oxide (6-deoxyandalusol, 10),[7] ent-19-hydroxy-13-epimanoyl oxide (11),[8] ent-kaur-16-en-19-ol (12),[9] and ent-kaur-16-en-19-oic acid (13),[10] in 0.0014, 0.003, 0.01, 0.0002, 0.003, 0.0001, and 0.001% yields, respectively, from the fresh tubers. After repeated separation of the 1-butanol-soluble portion by ordinary and reversed-phase silica gel column chromatography and

HPLC, followed by Sephadex LH-20 column chromatography, trifoliones B (2, 0.0003%), C (3, 0.001%), and D (4, 0.0003%), sagittariosides a (5, 0.0003%) and b (6, 0.0001%), and arabinothalictoside (7, 0.0006%) were isolated, together with daucosterin (0.002%), and daucosterin fatty acid ester (0.004%).[11]

Trifoliones A (1) and B (2) Trifolione A (1) was isolated as colorless needles of mp 106—108 °C. The IR spectrum of 1 showed absorption bands ascribable to hydroxyl, ketone, olefin, and vinyl functions at 3440, 1690, 1636, 920, 860 cm⁻¹. In the EI-MS of 1, a molecular

ion peak was observed at m/z 302, which was assigned as $C_{20}H_{30}O_2$ from the high-resolution MS measurement. The ^{1}H-NMR (CDCl$_3$) and ^{13}C-NMR (Table 1) spectra of 1 showed signals due to three tertiary methyls [δ 0.82 (s, 19-H$_3$), 0.88 (s, 20-H$_3$), 1.05 (br s, 17-H$_3$)], a hydroxyl-bearing methylene [δ 3.08, 3.57 (both d, $J=11$ Hz, 18-H$_2$)], a trisubstituted olefin [δ 5.31 (s, 14-H)], and a vinyl group [δ 4.91 (dd, $J=1$, 11 Hz), 4.92 (dd, $J=1$, 17 Hz) (16-H$_2$), 5.76 (dd, $J=11$, 17 Hz, 15-H)] together with six methylenes, two methines, three quaternary carbons, and a ketone group. These proton and carbon signals in the ^{1}H-NMR and ^{13}C-NMR spectra were assigned with the aid of homo and hetero correlation spectroscopy (^{1}H–^{1}H, ^{1}H–^{13}C COSY) and a distortionless enhancement by polarization transfer (DEPT) experiment. The connectivities of all quaternary carbons (C-2, 4, 8, 10, 13) in 1 were clarified by a correlation spectroscopy *via* long-range coupling (COLOC) NMR experiment with 1. Namely, long-range correlations were observed between the following carbons and protons of 1: 1-C and 20-H$_3$, 2-C and 1-H$_2$ [δ 2.23 (d, $J=13$ Hz), 2.39 (dd, $J=2$, 13 Hz)], 2-C and 3-H$_2$ [δ 2.01 (dd, $J=2$, 13 Hz), 2.74 (d, $J=13$ Hz)], 3-C and 19-H$_3$, 4-C and 3-H$_2$, 8-C and 7-H$_2$ [δ 2.14 (m), 2.32 (ddd, $J=2$, 5, 13 Hz)], 10-C and 20-H$_3$, 12-C and 17-H$_3$, 13-C and 14-H, 13-C and 15-H]. Furthermore, the relative stereostructure of 1 was characterized by a ^{1}H-NMR nuclear Overhauser and exchange spectroscopy (NOESY) experiment, which showed NOE correlations between the following proton pairs of 1: 5-H [δ 2.03 (br s)] and 9-H [δ 1.99 (dd, $J=3$, 12 Hz)], 5-H and 18-H$_2$, 11α-H [δ 1.44 (m)] and 17-H$_3$, 11α-H and 20-H$_3$, 14-H and 15-H, 18-H$_2$ and 19-H$_3$, 19-H$_3$ and 20-H$_3$ (as shown in Fig. 1). Comparisons of the ^{1}H-NMR and ^{13}C-NMR data for 1 with those for known diterpenes[12] led us to presume the ent-8 (14), 15-isopimaradien-18-ol structure of 1.

Acetylation of 1 with acetic anhydride in pyridine afforded the monoacetate (1a) which showed a molecular ion peak at m/z 344 in the EI-MS, and the molecular formula $C_{22}H_{32}O_3$ was determined by high-resolution MS measurement. The ^{1}H-NMR (CDCl$_3$) of 1a showed signals due to an acetoxy methylene group [δ 2.10 (3H, s), 3.63, 4.03 (both d, $J=11$ Hz, 18-H$_2$)] and, in the ^{13}C-NMR (Table 1) spectrum, an acetylation shift was observed around the C-18 position. Reduction of 1a with sodium borohydride (NaBH$_4$) in ethanol at room tem-

perature selectively afforded the 2-hydroxyl derivative (15). The IR spectrum of 15 showed absorption bands ascribable to hydroxyl, acetyl, olefin, and vinyl groups at 3450, 1740, 1260, 900, and 860 cm^{-1}. The ^{1}H-NMR (CDCl$_3$) and ^{13}C-NMR (Table 1) spectra of 15 showed signals due to a hydroxyl-bearing methine [δ 4.26 (dddd, $J=5$, 5, 5, 5 Hz, 2-H)], together with an acetoxyl-bearing methylene [δ 2.07 (s, OAc), 3.64, 3.86 (both d, $J=11$ Hz, 18-H$_2$)], three tertiary methyl, an olefin, and a vinyl groups. In the NOESY experiment on 15, NOE correlations were observed between 2-H and 18-H$_2$ together with the proton pairs shown in Fig. 1, so that the relative stereostructure of 15, including the configuration of the 2-hydroxyl group, was characterized.

Finally, the absolute stereostructure of 1 was clarified by application of a modified Mosher's method.[13] Thus, the signals due to protons on the C-1 and C-20 positions in the (+)-(R)-α-methoxy-α-(trifluoromethyl)phenylacetate (MTPA ester) (15b) appeared at higher fields than those of the (−)-(S)-MTPA ester (15b) ($\Delta\delta$ positive), while the signals due to protons attached to the C-3, C-18, and C-19 positions of 15b were observed at lower fields as compared to those of 15a ($\Delta\delta$ negative). Consequently, the absolute configuration at the C-2 position of 15 has been elucidated to be R and the absolute stereostructure of trifolione A has been determined to be ent-8 (14), 15-isopimaradiene-2-ket-18-ol (1). The CD (EtOH) data of 1 [$\Delta\varepsilon=-0.31$ (290 nm) (negative maximum), $\Delta\varepsilon=+1.98$ (210 nm) (positive maximum)] substantiated the absolute stereostructure of 1.

Trifolione C (3), obtained as a white powder, showed a quasimolecular ion peak at m/z 341 (M+Na)$^+$ in the positive FAB-MS, and the elemental composition $C_{20}H_{30}NaO_3$ was determined by high-resolution MS measurement. In the IR spectrum of 3, absorption bands due to hydroxyl, ketone, olefin, and vinyl groups were observed at 3350, 1700, 1635, 910, and 860 cm^{-1}. The ^{1}H-NMR (pyridine-d_5) spectrum of 3 showed signals assignable to two tertiary methyls [δ 1.02 (20-H$_3$), 1.07 (17-H$_3$)], two hydroxyl-bearing methylenes [δ 3.88, 4.07 (ABq, $J=11$ Hz, 19-H$_2$), 4.04 (s, 18-H$_2$)], a trisubstituted olefin [δ 5.35 (s, 14-H)], and a vinyl group [δ 5.00 (m, 16-H$_2$), 5.83 (dd, $J=11$, 17 Hz, 15-H)]. The ^{13}C-NMR (Table 1) spectrum of 3 was found to be very similar to that of 1 except for some signals affected by the 19-hydroxyl group. In the COLOC spectrum of 3, long-range

Chart 4
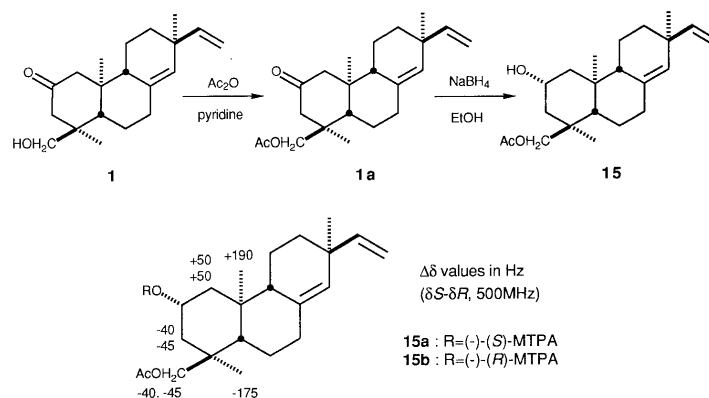
Table 1. The ^{13}C-NMR Data for **1**, **1a**, **2**, **3**, **4**, **15**, and the Aglycone Parts of **5** and **6**

	1	**1a**[a)	**2**[a)	**3**[b)	**4**[b)	**15**[a)	**5**[b)	**6**[b)
1	53.4	53.5	53.2	53.8	53.8	44.8	39.7	41.0
2	213.0	211.0	212.4	212.1	212.2	67.6	18.8	19.8
3	50.3	50.7	50.1	45.4	45.6	40.9	39.7	38.7
4	43.5	41.8	44.8[c)	48.4	47.7	34.5	37.3	43.9
5	46.1	47.9	38.6	47.5	41.2	47.5	57.4	57.0
6	22.5	22.8	28.4	23.2	31.1	22.5	20.7	23.0
7	34.1	34.2	72.7	36.2	72.2	35.6	44.1	42.6
8	135.6	135.2	138.0	136.6	140.1	136.2	76.0[c)	44.9
9	49.8	50.1	45.4	50.0	45.3	51.1	58.8	56.1
10	43.8	43.5	44.0[c)	43.1	43.4	37.6[c)	37.3	40.0
11	18.7	18.8	18.3	19.3	18.9	18.8	16.4	18.8
12	35.2	35.2	33.8	34.6	34.3	36.0	35.1	26.8
13	37.3	37.4	37.4	37.9	37.6	37.4[c)	73.3[c)	46.3
14	129.8	130.2	135.0	129.6	132.6	129.4	148.4	37.6
15	148.3	148.3	147.7	148.8	148.3	148.7	109.7	53.2
16	110.5	110.6	111.1	110.8	111.0	110.3	33.1	81.0
17	26.1	26.2	26.0	26.4	26.4	26.1	24.1	75.8
18	69.9	71.4	68.7	67.1	67.7	72.7	28.4	29.4
19	19.5	19.6	19.4	63.7	64.0	20.0	73.7	180.5
20	16.0	16.0	15.2	16.7	16.2	18.2	16.2	16.0

a, b) The spectra were measured in *a*) CDCl$_3$ or *b*) pyridine-d_5. *c*) Assignments may be interchangeable.
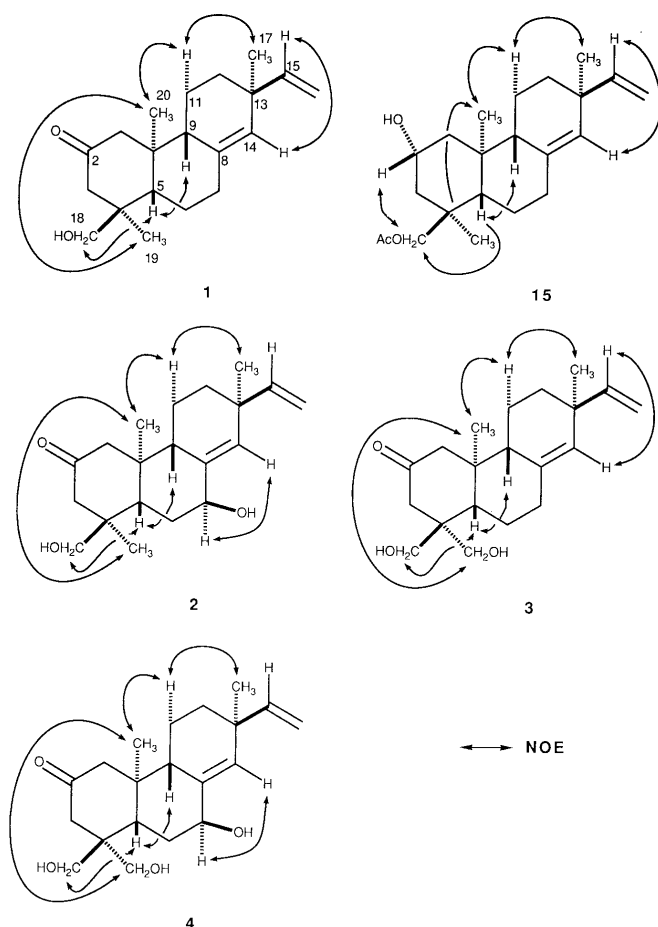
Fig. 1. NOE Correlations in the NOESY Spectra of **1**, **2**, **3**, **4**, and **15**

The stereostructure of **3** was confirmed by the NOESY experiment shown in Fig. 1 and the CD (EtOH) spectrum of **3** [$\Delta\varepsilon = -1.41$ (290 nm) (negative maximum), $\Delta\varepsilon = +2.73$ (220 nm) (positive maximum)] was found to show similar Cotton effects to those of **1**. Based on the above-mentioned evidence, the absolute stereostructure of trifolione C was determined to be *ent*-8 (**14**), 15-isopimara-diene-2-keto-18, 19-diol (**3**).

Trifoliones B (2) and D (4) Trifolione B (**2**) was also obtained as a white powder, and its molecular formula, $C_{20}H_{30}O_3$, identical with that of trifolione C (**3**), was determined by positive mode FAB-MS and high-resolution MS measurement. The IR spectrum of **2** showed similar absorption patterns to those of **1** and **3**. The ^{1}H-NMR (CDCl$_3$) and ^{13}C-NMR (Table 1) spectra of **2** showed signals assignable to a hydroxyl-bearing methine proton at δ 4.27 (dd, $J = 3$, 3 Hz, 7-H), together with the same functions as those of **1**: three tertiary methyls [δ 0.78 (19-H$_3$), 0.83 (20-H$_3$), 1.06 (17-H$_3$)], a hydroxyl-bearing methylene [δ 2.90, 3.62 (both d, $J = 14$ Hz, 18-H$_2$)], a trisubstituted olefin [δ 5.59 (d, $J = 1$ Hz, 14-H)], and a vinyl group [δ 4.95 (dd, $J = 1$, 17 Hz), 4.96 (dd, $J = 1$, 11 Hz) (16-H$_2$), 5.78 (dd, $J = 11$, 17 Hz, 15-H)].

Trifolione D (**4**) was isolated as colorless needles of mp 168—170 °C. The positive mode FAB-MS of **4** showed a quasimolecular ion peak at m/z 357 (M + Na)$^+$ and the molecular formula $C_{20}H_{30}O_4$ was clarified from the quasimolecular ion peak by high-resolution MS measurement. The ^{1}H-NMR (CDCl$_3$) and ^{13}C-NMR (Table 1) spectra of **4** showed signals due to a methine proton on carbon bearing a hydroxyl group [δ 4.29 (1H, br s, 7-H)], together with the same functions as those of **3**.

In the NOESY spectra of **2** and **4**, NOE correlations were observed between the following proton pairs of **2** (5-H and 18-H$_2$, 5-H and 9-H, 7-H and 14-H, 11α-H and 17-H$_3$, 11α-H and 20-H$_3$, 19-H$_3$ and 20-H$_3$) and **4** (5-H and 18-H$_2$, 5-H and 9-H, 7-H and 14-H, 11α-H and 17-H$_3$, 11α-H and 20-H$_3$, 19-H$_2$ and 20-H$_3$), as shown in Fig. 1. Based on above-mentioned evidence and a comparison of the CD spectra of **2** and **4** with those of **1** and **3**, it was concluded that **2** and **4** have the same stereostructure, including the absolute configuration, as **1** and **3**, respectively, except for the presence of the 7β-hydroxyl group. The configuration of the 7-hydroxyl group in **2** and **4** was substantiated by comparison of the ^{1}H–^{1}H couping constants for the 7α-proton of **3** and **4** with those for a known *ent*-isopimarane-type diterpene having a 7-hydroxyl group.[14) Furthermore, the absolute configuration of the 7β-hydroxyl group in **4** was determined by applying the excition chirality method[15) to the allylic benzoyl derivative of **4**. Namely, the 7-*O*-*p*-bromobenzoate (**16**) was prepared from **4** by introduction of the isopropylidene group at the 18,19-diol moiety with 2,2-dimethoxypropane and *p*-toluenesulfonic acid monohydrate, followed by *p*-bromobenzoylation of the 7-hydroxy group with *p*-bromobenzoic acid and dicyclohexylcarbodiimide (DCC) in the presence of dimethylaminopyridine (DMAP). Since a positive Cotton effect [$\Delta\varepsilon = +4.28$ (237 nm)] was observed in the CD spectrum of **15**, the 7S configuration of **4** was supported (Fig. 2). Consequently, the absolute stereostructures of trifoliones B and D were determined

correlations were observed between the following carbon and proton pairs: 1-C and 20-H$_3$, 2-C and 1-H$_2$ [δ 2.37, 2.57 (ABq, $J = 14$ Hz, 1-H$_2$)], 7-C and 14-H, 10-C and 20-H$_3$, 12-C and 17-H$_3$, 19-C and 5-H [δ 2.45 (dd-like)].

to be *ent*-8(14),15-isopimaradiene-2-keto-7β,18-diol (**2**) and *ent*-8(14),15-isopimaradiene-2-keto-7β,18,19-triol (**4**), respectively.

Sagittariosides a (5) and b (6) and Arabinothalictoside (7) Sagittariosides a (**5**) and b (**6**) were each obtained as a white powder and their positive FAB-MS showed quasimolecular ion peaks at *m/z* 491 (M+Na)$^+$ and *m/z* 475 (M+Li)$^+$ (from **5**) and at *m/z* 521 (M+Na)$^+$ and *m/z* 505 (M+Li)$^+$ (from **6**). The high-resolution MS measurement of **5** and **6** revealed their molecular formulae to be $C_{26}H_{44}O_7$ and $C_{26}H_{42}O_9$, respectively. Hydrolysis of **5** and **6** with β-glucosidase afforded *ent*-19-hydroxy-13-epimanoyl oxide (**11**) (from **5**) and *ent*-16α,17-hydroxy-kauran-19-oic acid (**14**)[10] (from **6**). The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra of **5** and **6**, showed signals due to a β-D-glucopyranosyl moiety [δ 4.86 (d, *J*=8 Hz, 1′-H) for **5** and at δ 5.05 (d, *J*=8 Hz, 1′-H) for **6**]. In the NOESY experiment on **6**, NOE correlation was observed between the anomeric proton (1′-H) and the 17-methylene protons. Furthermore, the ^{13}C-NMR data for **6** showed a glycosidation shift[16] around the C-17 position. Consequently, the structures of sagittariosides a (**5**) and b (**6**) were concluded to be as shown.

Arabinothalictoside (**7**) was also obtained as a white powder and its IR spectrum showed absorption bands ascribable to hydroxyl, aromatic ring, and nitro groups. The negative mode FAB-MS of **7** showed a quasimolecular ion peak at *m/z* 460 (M−H)$^-$, while a quasimolecular ion peak was observed at *m/z* 484 (M+Na)$^+$ in the positive mode FAB-MS. Here again, the molecular formula $C_{19}H_{27}NO_{12}$ of **7** was clarified from the quasimolecular ion peaks and high-resolution MS measurement. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (pyridine-d_5) spectra of **7** showed signals due to a β-D-glucopyranosyl moiety [δ 5.49 (d, *J*=8 Hz, 1′-H); δ$_C$ 102.3 (1′-C)], an α-L-arabinopyranosyl moiety [δ 4.98 (d,
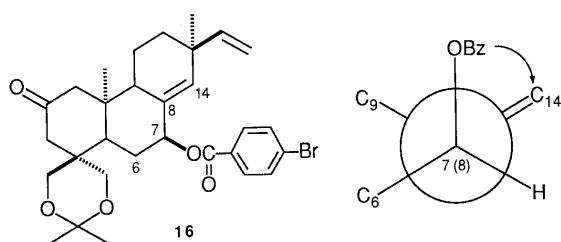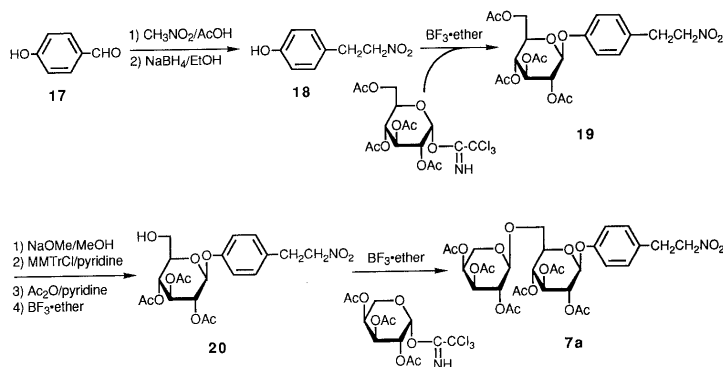
J=7 Hz, 1″-H); δ$_C$ 105.6 (1″-C)], and a 2-nitroethyl 4-phenoxyl moiety [δ 3.15, 4.76 (2H each, both t, *J*=7 Hz, 7, 8-H$_2$), 7.22, 7.38 (2H each, both d, *J*=9 Hz, 3, 5, 2, 6-H); δ$_C$ 32.6 (C-7), 76.6 (C-8), 117.5 (C-3, 5), 130.0 (C-1), 130.1 (C-2, 6), 157.7 (C-4)]. Consequently, the structure of arabinothalictoside (**7**) was determined. Finally, the structure of **7** was confirmed by synthesis from 4-hydroxy-2-nitroethylbenzene (**18**) which was prepared from *p*-hydroxybenzaldehyde (**17**) using a literature procedure.[17] Thus, glycosidation of **18** with *O*-(2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl)trichloroacetimidate[18] in dry CH_2CH_2 furnished 2′,3′,4′,6′-tetra-*O*-acetylthalictoside (**19**). Deacetylation of **19** furnished thalictoside,[17] which was subjected to monomethoxytritylation followed by acetylation and detritylation to yield 2′,3′,4′-tri-*O*-acetyl-thalictoside (**20**). Finally, glycosidation of **20** with *O*-(2,3,4-tri-*O*-acetyl-L-arabinopyranosyl)trichloroacetimidate gave **7a** which was identical with the hexaacetyl derivative of arabinothalictoside (**7**).

Inhibitory Effects of Trifoliones (1—4) and Related Diterpenes (8, 9, 11, 13) from Sagittaria trifolia L. Since the dried tuber of *Sagittaria trifolia* L. has been used to treat skin disease in Chinese traditional medicine, the principal components of this crude drug were expected to show antiallergic activity. Therefore, we examined the inhibitory effect of trifoliones (**1—4**) and related diterpene constituents isolated from the tuber of *Sagittaria trifolia* L. on histamine release from rat mast cells induced by compound 48/80 or calcium ionophore A-23187. As

Table 2. Inhibitory Effects of Torifoliones (**1—4**) and Related Diterpenes (**8, 9, 11, 13**) from *Sagittaria trilolia* on Histamine Release from Rat Mast Cells Induced by Compound 48/80 or Calcium Ionophore A-23187

	Compound 48/80	A-23187
Trifolione A (**1**)	43.1± 2.2	91.6±11.9
Trifolione B (**2**)	71.1± 5.8	85.6± 5.6
Trifolione C (**3**)	29.9±11.4	72.1± 8.3
Trifolione D (**4**)	24.5±11.0	78.1±20.5
Isoabienol (**8**)	0	30.2±14.9
13-Episclareol (**9**)	0	0
ent-19-Hydroxy-13-epimanoyl oxide (**11**)	0	0
ent-Kaur-16-en-19-oic acid (**13**)	0	0
DSCG	0	0
Tranilast	25.7± 5.2	0

Each value represents the mean with standard error of 3—5 experiments. The numeral values denote the inhibition % of histamine release at 10^{-4} M.

Fig. 2

Chart 5

summarized in Table 2, trifoliones (**1—4**) were found to exhibit more potent inhibitory activity than DSCG and tranilast on histamine release from cells induced by compound 48/80 and calcium ionophore A-23187. Isoabienol (**8**) also showed an inhibitory effect on histamine release induced by calcium ionophore A-23187, but the other diterpenes (**9, 11, 13**) did not possess this activity.

Experimental

The instruments used for obtaining physical data and the experimental conditions for chromatography were the same as described in our previous paper.[1]

Isolation of Trifoliones (1—4), Sagittariosides (5—6), Arabinothalictoside (7), and Known Compounds (8—13) from *Sagittaria trifolia* L. Fresh tubers of *Sagittaria trifolia* L. (8 kg, cultivated in Saitama Prefecture and purchased from Nijyo market, Kyoto) were cut finely and extracted with MeOH under reflux three times. Evaporation of the solvent from the extract under reduced pressure gave the MeOH extract (600 g). The MeOH extract was partitioned into AcOEt–H_2O mixture and the water-soluble portion was further extracted with *n*-BuOH. Removal of the solvent from the AcOEt-soluble and *n*-BuOH-soluble portions under reduced pressure yielded the AcOEt extract (24 g) and the *n*-BuOH extract (40 g).

The AcOEt extract (24 g) was subjected to silica gel column chromatography [*n*-hexane–AcOEt (5:1→3:1), AcOEt] followed by evaporation of the solvent under reduced pressure to furnish six fractions, fr. 1 (3.26 g, lipid fraction), fr. 2 (0.48 g), fr. 3 (0.53 g), fr. 4 (3.0 g), fr. 5 (4.5 g), fr. 6 (11.0 g), and 13-episclareol[6] (**9**, 0.80 g). Fraction 2 (0.48 g) was purified by repeated silica gel column chromatography [*n*-hexane–AcOEt (20:1) and benzene–AcOEt (50:1)] and recycling HPLC [SD-8 (column: Jaigel 1H-2H, solvent: $CHCl_3$, flow rate: 3 ml/min, Japan Analytical Ind. Co., Ltd.)] to give isoabienol[2] (**8**, 240 mg) and *ent*-13-epimanoyl oxide[7] (**10**, 16 mg). Purification by means of recycling HPLC (SD-8, the same conditions as in the case of fr. 2) of fr. 3 (0.2 g) afforded *ent*-kaur-16-en-19-ol[9] (**12**, 6.0 mg). Fraction 4 (3.0 g) was subjected to silica gel column chromatography [$CHCl_3$, $CHCl_3$–MeOH (5:1)] and reversed-phase silica gel column chromatography (70% aqueous MeOH) to afford *ent*-kaur-16-en-19-oic acid[10] (**13**, 80 mg) and *ent*-19-hydroxy-13-epimanoyl oxide[8] (**11**, 240 mg). Silica gel column chromatography [$CHCl_3$–MeOH (10:1), $CHCl_3$–AcOEt (5:1)] of fr. 5 (4.5 g) furnished trifolione A (**1**, 112 mg).

The *n*-BuOH-soluble extract (40 g) was subjected to silica gel column chromatography [$CHCl_3$–MeOH–H_2O (10:3:1→65:35:10, lower layer)] to give six fractions [fr. 1 (1.2 g), fr. 2 (1.2 g), fr. 3 (2.4 g), fr. 4 (0.56 g), fr. 5 (2.2 g), and fr. 6 (30 g, sugar fraction)]. Purification of fr. 1 (1.2 g) and fr. 2 (1.2 g) by silica gel column chromatography [$CHCl_3$–MeOH (10:1) and $CHCl_3$–MeOH–H_2O (10:3:1, lower layer)] followed by Sephadex LH-20 column chromatography to give daucosterin (160 mg) and daucosterin fatty acid ester (320 mg). Fraction 3 (2.4 g) was purified by silica gel [$CHCl_3$–MeOH–H_2O (10:3:1, lower layer)] and reversed-phase silica gel column chromatography (20% aqueous MeOH) and finally ODS-HPLC (column: Develosil ODS-5, solvent: 80% aqueous MeOH, flow rate: 1 ml/min) to give sagittarioside a (**5**, 24 mg), trifolione B (**2**, 24 mg), and C (**3**, 80 mg). Fraction 4 (0.56 g) was subjected to silica gel [$CHCl_3$–MeOH (10:1)] and reversed-phase silica gel column chromatography (60% aqueous MeOH) to give trifolione D (**4**, 24 mg). Silica gel column chromatography [$CHCl_3$–MeOH–H_2O (10:3:1, lower layer)] of fr. 5 (2.2 g) gave arabinothalictoside (**7**, 48 mg). Fraction 5 (2.2 g) was purified by ODS-HPLC (70% aqueous MeOH) to give sagittarioside b (**6**, 8.0 mg). The physical data for the known compounds (**8—13**) were identical with reported values.

Trifolione A (1): Colorless needles, mp 106—108 °C (from AcOEt–*n*-hexane), $[\alpha]_D^{20}$ −58.4° (*c*=0.25, $CHCl_3$). High-resolution EI-MS: Calcd for $C_{20}H_{30}O_2$ (M^+). 302.2244; Found: 302.2232. CD (*c*=0.15, EtOH): $\Delta\varepsilon$=−0.31 (290 nm, neg. max.), $\Delta\varepsilon$=+1.98 (210 nm, pos. max.). IR (KBr): 3440, 1690, 1636, 920, 860 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$, δ): 0.82 (3H, s, 19-H_3), 0.88 (3H, s, 20-H_3), 1.05 (3H, s, 17-H_3), 1.44 (1H, m, 14α-H), 1.99 (1H, dd, *J*=2, 13 Hz, 9-H), 2.01 (1H, d, *J*=2, 13 Hz, 3α-H), 2.03 (1H, br s, 5-H), 2.14 (1H, m, 7β-H), 2.23 (1H, d, *J*=13 Hz, 1β-H), 2.32 (1H, ddd, *J*=2, 5, 13 Hz, 7α-H), 2.39 (1H, dd, *J*=2, 13 Hz, 1α-H), 2.74 (1H, d, *J*=13 Hz, 3β-H), 3.08, 3.57 (ABq, *J*=11 Hz, 18-H_2), 4.91 (1H, dd, *J*=1, 11 Hz), 4.92 (1H, dd, *J*=1, 17 Hz)

(16-H_2), 5.31 (1H, br s, 14-H), 5.76 (1H, dd, *J*=11, 17 Hz, 15-H). ^{13}C-NMR (68.5 MHz, $CDCl_3$, δ_C): see Table 1. EI-MS *m/z*: 302 (M^+).

Trifolione B (2): A white powder, $[\alpha]_D^{20}$ +32.0° (*c*=0.20, $CHCl_3$). High-resolution FAB-MS: Calcd for $C_{20}H_{30}NaO_3$ $(M+Na)^+$: 341.2093. Found: 341.2101. CD (*c*=0.20, EtOH): $\Delta\varepsilon$=−1.35 (290 nm, neg. max.), $\Delta\varepsilon$=+4.91 (217 nm, pos. max.). IR (KBr): 3450, 1695, 1635, 910 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$, δ): 0.78 (3H, s, 19-H_3), 0.83 (3H, s, 20-H_3), 1.06 (3H, s, 17-H_3), 1.81 (1H, ddd, *J*=3, 3, 14 Hz, 6β-H), 1.94 (1H, dd, *J*=2, 13 Hz, 3α-H), 2.28 (1H, d, *J*=13 Hz, 1β-H), 2.36 (1H, dd, *J*=2, 13 Hz, 1α-H), 2.45 (1H, br s, 5-H), 2.46 (1H, dd, *J*=3, 13 Hz, 9-H), 2.92 (1H, d, *J*=13 Hz, 3β-H), 2.90, 3.62 (1H, both d, *J*=14 Hz, 18-H_2), 4.27 (1H, dd, *J*=3, 3 Hz, 7-H), 4.95 (1H, dd, *J*=1, 17 Hz), 4.96 (1H, dd, *J*=1, 11 Hz) (16-H_2), 5.59 (1H, d, *J*=1 Hz, 14-H), 5.78 (1H, dd, *J*=11, 17 Hz, 15-H). ^{13}C-NMR (67.5 MHz, $CDCl_3$, δ_C): see Table 1. Positive mode FAB-MS *m/z*: 341 $(M+Na)^+$, 325 $(M+Li)^+$.

Trifolione C (3): A white powder, $[\alpha]_D^{20}$ −13.5° (*c*=0.65, MeOH). High-resolution FAB-MS: Calcd for $C_{20}H_{30}NaO_3$ $(M+Na)^+$: 341.2093. Found: 341.2114. CD (*c*=0.024, EtOH): $\Delta\varepsilon$=−1.41 (290 nm, neg. max.), $\Delta\varepsilon$=+2.73 (220 nm, pos. max.). IR (KBr): 3350, 1700, 1635, 910, 860 cm^{-1}. ^{1}H-NMR (270 MHz, C_5D_5N, δ): 1.02 (3H, s, 20-H_3), 1.07 (3H, s, 17-H_3), 2.34 (1H, m, 7α-H), 2.37, 2.57 (ABq, *J*=14 Hz, 1-H_2), 2.45 (1H, dd-like, 5-H), 3.03, 3.09 (ABq, *J*=14 Hz, 3β, 3α-H), 3.88, 4.07 (2H, ABq, *J*=11 Hz, 19-H_2), 4.04 (2H, s, 18-H_2), 5.00 (2H, m, 16-H_2), 5.35 (1H, s, 14-H), 5.83 (1H, dd, *J*=11, 17 Hz, 15-H). ^{13}C-NMR (67.5 MHz, C_5D_5N, δ_C): see Table 1. Positive mode FAB-MS *m/z*: 341 $(M+Na)^+$, 325 $(M+Li)^+$.

Trifolione D (4): Colorless needles, mp 168—170 °C (from MeOH–H_2O), $[\alpha]_D^{20}$ +18.4° (*c*=1.10, MeOH). High-resolution FAB-MS: Calcd for $C_{20}H_{30}NaO_4$ $(M+Na)^+$: 357.2042. Found: 357.2055. CD (*c*=0.02, EtOH): $\Delta\varepsilon$=−2.23 (290 nm, neg. max.), $\Delta\varepsilon$=+4.76 (216 nm, pos. max.). IR (KBr): 3450, 1690, 1640, 910 cm^{-1}. ^{1}H-NMR (270 MHz, C_5D_5N, δ): 1.07 (3H, s, 17-H_3), 1.09 (3H, s, 20-H_3), 2.06 (1H, ddd, *J*=3, 10, 10 Hz, 6α-H), 2.45 (1H, d, *J*=14 Hz, 1α-H), 2.48 (1H, m, 6β-H), 2.60 (1H, d, *J*=14 Hz, 1β-H), 3.03 (1H, d, *J*=14 Hz, 3β-H), 3.16 (1H, d, *J*=14 Hz, 3α-H), 3.21 (1H, dd-like, 5-H), 3.96, 4.14 (ABq, *J*=10 Hz, 19-H_2), 4.06, 4.14 (ABq, *J*=10 Hz, 18-H_2), 4.29 (1H, br s, 7-H), 4.92 (1H, dd, *J*=1, 10 Hz), 4.98 (1H, dd, *J*=1, 17 Hz) (16-H_2), 5.63 (1H, s, 14-H), 5.75 (1H, dd, *J*=10, 17 Hz, 15-H). ^{13}C-NMR (67.5 MHz, C_5D_5N, δ_C): see Table 1. Positive mode FAB-MS *m/z*: 357 $(M+Na)^+$.

Sagittarioside a (5): A white powder, $[\alpha]_D^{20}$ −35.7° (*c*=0.75, MeOH). High-resolution FAB-MS: Calcd for $C_{26}H_{44}NaO_7$ $(M+Na)^+$: 491.2984. Found: 491.2995. IR (KBr): 3400, 1640, 1035, 925 cm^{-1}. ^{1}H-NMR (270 MHz, C_5D_5N, δ): 0.80 (3H, s, 20-H_3), 1.02 (1H, m, 5-H), 1.18 (1H, m, 9-H), 1.20 (3H, s, 18-H_3), 1.23 (3H, s, 16-H_3), 1.31 (3H, s, 17-H_3), 3.64 (1H, d, *J*=12 Hz), 4.23 (1H, m) (19-H_2), 4.41 (1H, dd, *J*=5, 12 Hz), 4.58 (1H, dd, *J*=2, 12 Hz) (6'-H_2), 4.86 (1H, d, *J*=8 Hz, 1'-H), 4.95 (1H, d, *J*=18 Hz), 5.00 (1H, d, *J*=11 Hz) (15-H_2), 6.10 (1H, dd, *J*=11, 18 Hz, 14-H). ^{13}C-NMR (67.5 MHz, C_5D_5N, δ_C): 62.9 (C-6'), 71.8 (C-4') 75.3 (C-2'), 78.5 (C-5'), 78.9 (C-3'), 105.5 (C-1'), and other signals as shown in Table 1. Positive mode FAB-MS *m/z*: 491 $(M+Na)^+$, 475 $(M+Li)^+$.

Sagittarioside b (6): A white powder, $[\alpha]_D^{20}$ −28.7° (*c*=0.23, MeOH). High-resolution FAB-MS: Calcd for $C_{26}H_{42}NaO_9$ $(M+Na)^+$: 521.2726. Found: 521.2737. IR (KBr): 3500, 1695, 1260, 1080 cm^{-1}. ^{1}H-NMR (270 MHz, C_5D_5N, δ): 1.15 (3H, s, 20-H_3), 1.35 (3H, s, 18-H_3), 4.52, 4.95 (2H, both d, *J*=11 Hz, 17-H_2), 5.05 (1H, d, *J*=8 Hz, 1'-H). ^{13}C-NMR (67.5 MHz, C_5D_5N, δ_C): 62.8 (C-6'), 71.7 (C-4'), 75.6 (C-2'), 78.6 (C-5'), 78.8 (C-3'), 106.7 (C-1'), and other signals as shown in Table 1. Positive mode FAB-MS *m/z*: 521 $(M+Na)^+$, 505 $(M+Li)^+$.

Arabinothalictoside (7): A white powder, $[\alpha]_D^{20}$ −51.3° (*c*=0.76, MeOH). High-resolution FAB-MS: Calcd for $C_{19}H_{27}NNaO_{12}$ $(M+Na)^+$: 484.1436. Found: 484.1426. IR (KBr): 3300, 1550, 1380 cm^{-1}. UV λ_{max}^{MeOH} (ε): 271 (2620), 220 (9355). ^{1}H-NMR (270 MHz, C_5D_5N, δ): 3.15 (2H, t, *J*=7 Hz, 7-H_2), 4.76 (2H, t, *J*=7 Hz, 8-H_2), 4.98 (1H, d, *J*=7 Hz, 1''-H), 5.49 (1H, d, *J*=8 Hz, 1'-H), 7.22 (2H, d, *J*=9 Hz, 3, 5-H), 7.38 (2H, d, *J*=9 Hz, 2, 6-H). ^{13}C-NMR (67.5 MHz, C_5D_5N, δ_C): 32.6 (C-7), 66.9 (C-5''), 69.5 (C-6'), 71.0 (C-4''), 71.2 (C-4'), 74.7 (C-2''), 74.9 (C-2'), 76.6 (C-8), 77.6 (C-3''), 78.0 (C-5'), 78.3 (C-3'), 102.3 (C-1'), 105.6 (C-1''), 117.5 (C-3, 5), 130.0 (C-1), 130.1 (C-2, 6), 157.7 (C-4). Positive mode FAB-MS *m/z*: 484 $(M+Na)^+$. Negative mode FAB-MS *m/z*: 460 $(M-H)^-$.

Acetylation of Trifolione A (1) A solution of **1** (20 mg) in pyridine (1.5 ml) was treated with Ac_2O (1.0 ml) and the whole mixture was stirred at 20 °C for 3 h. It was poured into ice-water and the whole was extracted

with AcOEt. The AcOEt extract was washed with saturated aqueous NaHCO$_3$ and brine, then dried over MgSO$_4$ powder. Removal of the solvent from the AcOEt extract under reduced pressure gave a residue, which was purified by silica gel column chromatography [n-hexane–AcOEt (4:1)] to give the monoacetate (1a, 20 mg).

1a: Colorless oil, $[\alpha]_D^{20}$ $-43.8°$ ($c=0.21$, CHCl$_3$). High-resolution EI-MS: Calcd for C$_{22}$H$_{32}$O$_3$ (M$^+$): 344.2350. Found: 344.2356. IR (KBr): 1740, 1715, 1635, 1270, 915 cm^{-1}. ^{1}H-NMR (270 MHz, CDCl$_3$, δ): 0.88 (3H, s, 19-H$_3$), 0.92 (3H, s, 20-H$_3$), 1.04 (3H, s, 17-H$_3$), 2.10 (3H, s, acetyl methyl), 2.14 (1H, dd, $J=2$, 14 Hz), 2.57 (1H, d, $J=14$ Hz) (3-H$_2$), 2.22 (1H, d, $J=13$ Hz), 2.41 (1H, dd, $J=2$, 13 Hz) (1-H$_2$), 3.63, 4.03 (both d, $J=11$ Hz, 18-H$_2$), 4.88 (2H, m, 16-H$_2$), 5.31 (1H, s, 14-H), 5.75 (1H, dd, $J=11$, 17 Hz, 15-H). ^{13}C-NMR (67.5 MHz, CDCl$_3$, δ_C): see Table 1. EI-MS m/z: 344 (M$^+$).

Reduction of Trifolione A Monoacetate (1a) A solution of 1a (20 mg) in EtOH (1 ml) was treated with NaBH$_4$ (3.7 mg) and the reaction mixture was stirred at 20 °C for 30 min. It was neutralized with Dowex HCR W × 2 (H$^+$ form) and then filtered to remove the resin. Removal of the solvent from the filtrate under reduced pressure furnished 15 (17 mg).

15: A white powder, $[\alpha]_D^{20}$ $-16.0°$ ($c=0.05$, CHCl$_3$). High-resolution EI-MS: Calcd for C$_{22}$H$_{34}$O$_3$ (M$^+$): 346.2506. Found: 346.2517. IR (KBr): 3450, 1740, 1260, 900, 860 cm^{-1}. ^{1}H-NMR (270 MHz, CDCl$_3$, δ): 1.05 (3H, s, 17-H$_3$), 1.08 (3H, s, 20-H$_3$), 1.10 (3H, s, 19-H$_3$), 1.30 (1H, m, 5-H), 1.48, 1.87 (2H, m, 1-H$_2$), 1.63 (2H, m, 3-H$_2$), 1.73 (1H, m, 9-H), 2.07 (3H, s, acetyl methyl), 3.64, 3.86 (1H, both d, $J=11$ Hz, 18-H$_2$), 4.26 (1H, dddd, $J=5$, 5, 5, 5 Hz, 2-H), 4.90 (1H, d, $J=11$ Hz), 4.92 (1H, d, $J=17$ Hz) (16-H$_2$), 5.26 (1H, s, 14-H), 5.76 (1H, dd, $J=11$, 17 Hz, 15-H). ^{13}C-NMR (67.5 MHz, CDCl$_3$, δ_C): see Table 1. EI-MS m/z: 346 (M$^+$).

Preparation of the (−)-(S)-MTPA Ester (15a) from 15 A solution of 15 (3.0 mg) in CH$_2$Cl$_2$ (0.4 ml) was treated with (−)-(S)-MTPA (13.4 mg) in the presence of DCC (13.8 mg) and DMAP (1.5 mg), and the reaction mixture was stirred at 20 °C under an N$_2$ atmosphere for 15 min. It was poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner gave a product which was purified by preparative TLC [n-hexane–AcOEt (4:1)] to furnish 15a (5 mg).

15a: A white powder. ^{1}H-NMR (500 MHz, CDCl$_3$, δ): 0.54 (3H, s, 19-H$_3$), 0.86 (3H, s, 20-H$_3$), 1.03 (3H, s, 17-H$_3$), 1.37 (1H, m, 5-H), 1.49 (1H, dd, $J=4$, 15 Hz, 1β-H), 1.61 (1H, ddd, $J=4$, 4, 15 Hz, 3α-H), 1.70 (1H, dd, $J=4$, 15 Hz, 3β-H), 2.02 (1H, ddd, $J=4$, 4, 15 Hz, 1α-H), 3.50, 3.79 (2H, both d, $J=12$ Hz, 18-H$_2$), 4.88 (dd, $J=1$, 11 Hz), 4.89 (dd, $J=1$, 17 Hz) (16-H$_2$), 5.25 (1H, s, 14-H), 5.53 (1H, q, $J=5$ Hz, 2-H), 5.75 (1H, dd, $J=11$, 17 Hz, 15-H), 7.36—7.40 (4H, m).

Preparation of the (+)-(R)-MTPA Ester (15b) from 15 A solution of 15 (3.0 mg) in CH$_2$Cl$_2$ (0.5 ml) was treated with (+)-(R)-MTPA (13.4 mg) in the presence of DCC (13.8 mg) and DMAP (1.5 mg), and the reaction mixture was stirred at 20 °C under an N$_2$ atmosphere for 15 min. It was worked up as described above to give an AcOEt extract, which was purified by preparative TLC [n-hexane–AcOEt (4:1)] to furnish 15b (4.5 mg).

15b: A white powder. ^{1}H-NMR (500 MHz, CDCl$_3$, δ): 0.48 (3H, s, 20-H$_3$), 0.89 (3H, s, 19-H$_3$), 0.98 (3H, s, 17-H$_3$), 1.36 (1H, m, 5-H), 1.39 (1H, dd, $J=4$, 15 Hz, 1β-H), 1.68 (1H, m, 9-H), 1.68 (1H, m, 9-H), 1.70 (1H, ddd, $J=4$, 4, 15 Hz, 3α-H), 1.78 (1H, dd, $J=4$, 15 Hz, 3β-H), 1.92 (1H, ddd, $J=4$, 4, 15 Hz, 1α-H), 2.07 (3H, s, OAc), 3.59, 3.87 (1H, both d, $J=12$ Hz, 18-H$_2$), 4.87 (1H, dd, $J=2$, 11 Hz), 4.89 (1H, dd, $J=2$, 17 Hz) (16-H$_2$), 5.23 (1H, s, 14-H), 5.53 (1H, q, $J=5$ Hz, 2-H), 5.74 (1H, dd, $J=11$, 17 Hz, 15-H), 7.36—7.40 (6H, m), 7.54 (4H, m).

Preparation of Trifolione D 7-O-p-Bromobenzoate (16) A solution of trifolione D (4, 3 mg) in dry DMF (0.2 ml) was treated with 2,2-dimethoxypropane (150 μl) and p-TsOH (1.5 mg), and the reaction mixture was stirred at 20 °C under an N$_2$ atmosphere for 5 min. It was poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner gave the crude product, which was purified on an SiO$_2$ column [n-hexane–AcOEt (1:1)] to furnish the 18, 19-acetonide (3 mg), colorless oil. ^{1}H-NMR (270 MHz, CDCl$_3$, δ): 0.72 (3H, s, 20-H$_3$), 1.05 (3H, s, 17-H$_3$), 1.33, 1.36 (3H, both s, isopropylidene methyl), 2.95 (1H, dd, $J=2$, 14 Hz, 3β-H), 3.35, 3.85 (1H, both d, $J=12$ Hz, 19-H$_2$), 3.50, 3.88 (1H, both d, $J=12$ Hz, 18-H$_2$), 4.29 (1H, m, 7-H), 4.94 (1H, d, $J=17$ Hz), 4.96 (1H, d, $J=11$ Hz) (16-H$_2$), 5.61 (1H, s, 14-H), 5.77 (1H, dd, $J=11$, 17 Hz, 15-H).

A solution of the 18, 19-acetonide (3 mg) in dry CH$_2$Cl$_2$ (0.3 ml) was treated with p-bromobenzoic acid (24 mg) in the presence of DCC (24 mg) and DMAP (8.8 mg), and the reaction mixture was stirred at 20 °C under an N$_2$ atmosphere for 1.5 h, and 40 °C for 5 min. It was poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner and purification of the crude products on an SiO$_2$ column [n-hexane–AcOEt (4:1)] furnished 16 (3 mg), colorless oil. ^{1}H-NMR (270 MHz, CDCl$_3$, δ): 0.79 (3H, s, 20-H$_3$), 1.06 (3H, s, 17-H$_3$), 1.30, 1.33 (3H, both s, isopropylidene methyl), 2.90 (1H, dd, $J=3$, 14 Hz, 3β-H), 3.33, 3.69 (1H, both d, $J=12$ Hz, 19-H$_2$), 3.49, 3.81 (1H, both d, $J=12$ Hz, 18-H$_2$), 4.89 (1H, d, $J=18$ Hz), 4.90 (1H, d, $J=11$ Hz) (16-H$_2$), 5.66 (1H, m, 7-H), 5.69 (1H, dd, $J=11$, 18 Hz, 15-H), 5.89 (1H, s, 14-H), 7.68, 7.86 (2H, both d, $J=7$ Hz, p-bromobenzoate part). CD ($c=0.15$, EtOH): $\Delta\varepsilon = -2.03$ (295 nm, neg. max.), $\Delta\varepsilon = +4.28$ (237 nm, pos. max.).

Enzymatic Hydrolysis of Sagittarioside a (5) A solution of sagittarioside a (5, 3 mg) in acetate buffer (1 ml) was treated with β-glucosidase (10 mg), and the mixture was stirred at 37 °C for 80 h. After removal of the solvent from the reaction mixture under reduced pressure, the residue was purified by silica gel column chromatography (CHCl$_3$) to furnish 19-hydroxy-13-epimanoyl oxide (1.0 mg).

19-Hydroxy-13-epimanoyl oxide (11) thus obtained was shown to be identical with an authentic sample from Sagittaria trifolia, based on comparisons of the $[\alpha]_D$ data and TLC behavior.

Enzymatic Hydrolysis of Sagittarioside b (6) A solution of sagittarioside b (6, 3 mg) in acetate buffer (1 ml) was treated with β-glucosidase (10 mg), and the mixture was stirred at 37 °C for 48 h. After removal of the solvent, the product was purified by silica gel column chromatography [CHCl$_3$–MeOH (1:1)] furnish 16α,17-dihydroxy-ent-kauran-19-oic acid (14, 1.6 mg). The $[\alpha]_D$ and NMR data of 16α,17-dihydroxy-ent-kauran-19-oic acid thus obtained were identical with reported values.[10]

Glycosidation of p-Hydroxynitroethylbenzene (18) A solution of 18 (180 mg) which was prepared from 17 using a literature precedure[18] in dry CH$_2$Cl$_2$ (5 ml) in the presence of molecular sieves-4A (1 g) was treated with 1-imidate-2,3,4,6-tetra-O-acetyl-D-glucopyranose (1.06 g) and the mixture was stirred at 20 °C under an N$_2$ atmosphere for 10 min. It was treated with BF$_3$·Et$_2$O (280 μl), and stirring was continued for 5 h. The reaction mixture was poured into ice-water and the whole was extracted with CH$_2$Cl$_2$. The CH$_2$Cl$_2$ extract was washed with brine, then dried over MgSO$_4$. After work-up of the CH$_2$Cl$_2$ extract in the usual manner, the crude product was purified by silica gel column chromatography [CHCl$_3$–acetone (50:1)] to furnish 19 (480 mg), colorless oil. ^{1}H-NMR (270 MHz, CDCl$_3$, δ): 2.04, 2.05, 2.06, 2.08 (3H, all s, acetyl methyl), 3.27 (2H, t, $J=7$ Hz, 7-H$_2$), 3.86 (1H, ddd, $J=2$, 5, 10 Hz, 5'-H), 4.16 (dd, $J=2$, 12 Hz), 4.28 (dd, $J=5$, 12 Hz) (6'-H$_2$), 4.58 (2H, t, $J=7$ Hz, 8-H$_2$), 5.06 (1H, d, $J=8$ Hz, 1'-H), 5.23 (3H, m, 2', 3', 4'-H), 6.74 (2H, d, $J=9$ Hz, 3, 5-H), 7.14 (2H, d, $J=9$ Hz, 2, 6-H).

Acetylation of Arabinothalictoside (7) A solution of 7 (27.3 mg) in pyridine (2 ml) was treated with Ac$_2$O (2 ml) and the mixture was stirred at 20 °C for 12 h, then poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner gave a crude product, which was purified by silica gel column chromatography [n-hexane–AcOEt (1:1)] to give the hexaacetate (7a, 20 mg).

7a: A colorless oil, $[\alpha]_D^{20}$ $-26.6°$ ($c=1.15$, MeOH). ^{1}H-NMR (270 MHz, CDCl$_3$, δ): 1.91, 2.02, 2.03 (3H, all s), 2.05 (9H, s) (acetyl methyl), 3.30 (2H, t, $J=7$ Hz, 7-H$_2$), 3.22 (1H, dd, $J=9$, 12 Hz), 4.08 (1H, dd, $J=5$, 12 Hz) (6''-H$_2$), 3.67 (1H, dd, $J=7$, 12 Hz), 3.80 (1H, m) (6'-H$_2$), 4.54 (1H, d, $J=7$ Hz, 1''-H), 4.63 (2H, t, $J=7$ Hz, 8-H$_2$), 5.00 (1H, d, $J=8$ Hz, 1'-H), 6.95 (2H, d, $J=9$ Hz, 3, 5-H), 7.20 (2H, d, $J=9$ Hz, 2, 6-H). ^{13}C-NMR (67.5 MHz, C$_5$D$_5$N, δ_C): 20.4, 20.5 (acetyl methyl), 32.6 (C-7), 62.3 (C-5''), 67.6 (C-6'), 69.2 (C-4''), 69.4 (C-4'), 71.4 (C-2''), 71.8 (C-2'), 72.2 (C-3''), 73.4 (C-3') 74.3 (C-5'), 76.5 (C-8), 99.1 (C-1'), 101.0 (C-1''), 117.5 (C-3, 5), 130.5 (C-2, 6), 131.5 (C-1), 156.7 (C-4), 169.7, 169.8, 170.0, 170.3 (acetyl carbonyl).

Preparation of 2,3,4-Tri-O-acetylthalictoside (20) A solution of 19 (300 mg) in MeOH (10 ml) was treated with 10% aqueous K$_2$CO$_3$ (30 ml) and the mixture was stirred at 20 °C for 15 min. The reaction mixture was neutralized with IRC-76 (H$_3$O$^+$) and then filtered. After removal of the solvent from the filtrate under reduced pressure, a residue was purified by silica gel column chromatography [CHCl$_3$–MeOH (5:1)] to furnish thalictoside[8] (200 mg), which was identified by comparison of its physical data with reported values.

A solution of thalictoside (300 mg) in dry pyridine (0.5 ml) was treated

with MMTrCl (77 mg), and the mixture was stirred at 30 °C under an N_2 atmosphere for 3 h. It was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed with diluted aqueous HCl, aqueous saturated $NaHCO_3$, and brine, and then dried over $MgSO_4$. After removal of the solvent from the AcOEt extract under reduced pressure, the product was purified by silica gel column chromatography [$CHCl_3$–MeOH (20:1)] to furnish 6'-O-MMTr-thalictoside (136 mg), a colorless oil. ^{1}H-NMR (270 MHz, $CDCl_3$, δ): 3.26 (2H, t, $J=7$ Hz, 7-H_2), 3.65 (3H, s, methoxyl), 4.83 (2H, t, $J=7$ Hz, 8-H_2), 5.63 (1H, d, $J=8$ Hz, 1'-H), 6.86 (2H, d, $J=9$ Hz, 3, 5-H), 7.24 (2H, d, $J=9$ Hz, 2, 6-H), 7.22—8.72 (all 14H, triphenyl).

A solution of 6'-O-MMTr-thalictoside (20 mg) in pyridine (0.3 ml) was treated with Ac_2O (0.2 ml) and the whole was stirred at room temperature under an N_2 atmosphere for 12 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. After work-up of the AcOEt extract, the residue was purified by silica gel column chromatography [n-hexane–AcOEt (1:1)] to give 2',3',4'-triacetyl-6'-MMTr-thalictoside (20 mg), a colorless oil. ^{1}H-NMR (270 MHz, $CDCl_3$, δ): 2.00, 2.01, 2.07 (3H, all s, acetyl methyl), 3.26 (2H, t, $J=7$ Hz, 7-H_2), 3.78 (3H, s, methoxyl), 4.55 (2H, t, $J=7$ Hz, 8-H_2), 6.80 (2H, d, $J=9$ Hz, 3, 5-H), 7.28 (2H, d, $J=9$ Hz, 2, 6-H), 7.10—7.47 (all 14H, triphenyl).

A solution of 2',3',4'-triacetyl-6'-MMTr-thalictoside (20 mg) in dry THF–Et_2O (1:2, 2 ml) was treated with $BF_3 \cdot Et_2O$ (0.8 ml) and the mixture was stirred at room temperature under an N_2 atmosphere for 20 min. It was poured into AcOEt and the whole was extracted with AcOEt. The AcOEt extract was washed with aqueous saturated $NaHCO_3$ and brine. After work-up of the AcOEt extract, a residue was purified by silica gel column chromatography [n-hexane–AcOEt (2:3)] to give 2',3',4'-triacetyl-thalictoside (**20**, 13 mg), a colorless oil. ^{1}H-NMR (270 MHz, $CDCl_3$, δ): 2.04, 2.05, 2.07 (3H, all s, acetyl methyl), 3.27 (2H, t, $J=7$ Hz, 7-H_2), 3.75 (3H, m, 5', 6'-H), 4.58 (2H, t, $J=7$ Hz, 8-H_2), 5.07—5.37 (4H, all m, 1', 2', 3', 4'-H), 6.93 (2H, d, $J=9$ Hz, 3, 5-H), 7.14 (2H, d, $J=9$ Hz, 2, 6-H).

Glycosidation of 20 In the presence of molecular sieves 4A (0.1 g), a solution of 2',3',4'-triacetyl-thalictoside (**20**, 20 mg) in dry CH_2Cl_2 (0.5 ml) was treated with 2,3,4-triacetyl-L-arabinopyranosyl-1-O-trichloroacetaimidate (55.5 mg) and $BF_3 \cdot Et_2O$ (20 μl). The mixture was stirred at room temperature for 40 min, and poured into ice-water. The whole was extracted with CH_2Cl_2, and the CH_2Cl_2 extract was washed with brine. After work-up of the CH_2Cl_2 extract, a residue was purified by silica gel column chromatography [n-hexane–AcOEt (1:1)] to give arabinothalictoside-hexaacetate (**7a**, 21 mg), which was identified on basis of [α]$_D$ (MeOH), TLC [n-hexane–AcOEt (1:1)]. ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$) comparisons with the hexaacetate of natural arabinothalictoside.

Histamine Release from Rat Peritoneal Mast Cells The method of bioassay was essentially as described in the previous report.[4] Male Wistar rats (Kiwa Laboratory Animals Ltd.) weighing 300—400 g were exsanguinated and injected intraperitoneally with 10 ml of physiological solution consisting of NaCl (154 mM), KCl (2.7 mM), $CaCl_2$ (0.9 mM), glucose (5.6 mM), and HEPES (Dotite, 5 mM, pH 7.4). The abdominal region was gently massaged for 2 min and then the peritoneal exudate was collected. Mast cells were obtained from the peritoneal exudate according to the method of Nemeth and Rohlich.[19] The cell suspension from the exudate was mixed with various concentrations of test compounds and the mixture was preincubated at 37 °C for 15 min. Then compound 48/80 or calcium ionophore A-23187 was added and the mixture was incubated at 37 °C for 10 min. After cooling, the amount of histamine released into the reaction mixture was measured by the quantitative fluorescence method.[20] The results are summarized in Table 2.

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