ABSOLUTE STEREOSTRUCTURES OF TRIFOLIONES A, B, C, AND D, NEW BIOLOGICALLY ACTIVE DITERPENES FROM THE TUBER OF *SAGITTARIA TRIFOLIA* L.

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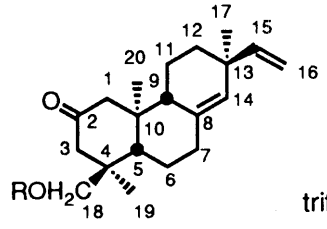
Four new biologically active diterpenes, trifoliones A, B, C, and D, were isolated from the tuber of *Sagittaria trifolia* L. together with three new glycosides, sagittariosides a and b and arabinothalictoside. Their stereostructures were determined on the basis of chemical and physicochemical evidence which included the application of a modified Mosher's method and an exciton chirality method. Trifoliones A, B, C, and D exhibited inhibitory effects on the histamine release from rat mast cells induced by compound 48/80 or calcium ionophore A-23187.

KEYWORDS *Sagittaria trifolia* ; Alismataceae ; aquatic plant ; trifolione ; sagittarioside ; arabinothalictoside

The tuber of aquatic plant *Sagittaria trifolia* L. (Alismataceae, Kuwai in Japanese) is known as a garnish foodstuff in Japanese-style dishes. In Chinese traditional medicine, the tuber of *Sagittaria trifolia* has been used medicinally during childbirth and for skin diseases. In regard to the chemical constituent of this crude drug, isoabienol was isolated from the Japanese fresh tuber.[1] As a part of our studies on antiallergic constituents of foodstuffs,[2] we have isolated four new biologically active diterpenes named trifoliones A (**1**), B (**2**), C (**3**), and D (**4**) together with two new diterpene glucosides, sagittariosides a (**6**) and b (**7**), and a phenolic glycoside containing a nitro group, arabinothalictoside (**8**), from the fresh tuber of *Sagittaria trifolia*. This paper deals with the structure elucidations of trifoliones A-D (**1**~**4**) which exhibited inhibitory effects on the histamine release from rat mast cells. In addition, three new glycosides, **6**, **7**, and arabinothalictoside (**8**) were chemically elucidated.

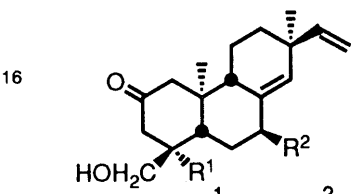
The MeOH extract of the tuber cultivated in Saitama Prefecture was partitioned into an AcOEt-water mixture and the water-soluble portion was further extracted with 1-BuOH. Repeated separation of the AcOEt-soluble portion by normal and reversed phase SiO_2 column chromatography and HPLC (JAIGEL 1H-2H) furnished **1** (0.0014% from the fresh tuber) together with isoabienol[1] (0.003%), sclareol[3] (0.01%), *ent*-kaur-16-en-19-oic acid[4] (0.001%), *ent*-19-hydroxy-13-epi-manoyl oxide[5] (0.003%), *ent*-13-epi-manoyl oxide[6] (0.0002%), and *ent*-kaur-16-en-19-ol [7] (0.001%). From the 1-BuOH-soluble portion by use of normal and reversed phase SiO_2, Sephadex LH-20 column chromatography, and HPLC (ODS), **2** (0.0001%), **3** (0.0003%), and **4** (0.0003%) were isolated with **6** (0.0003%), **7** (0.0001%), arabinothalictoside (**8**, 0.0006%), and 8α, 13β-dihydroxy-labd-14-en-3β-*O*-β-D-glucopyranoside (0.0003%).[3]

Trifolione A (**1**), colorless needles, mp 106-108°C (from AcOEt-*n*-hexane), $[\alpha]_D$ -58.4° ($CHCl_3$), $C_{20}H_{30}O_2$, showed absorption bands due to hydroxyl (3440 cm^{-1}), ketone (1690 cm^{-1}), olefin and vinyl (920, 860 cm^{-1}) functions in its IR spectrum. The ^1H (500 MHz) and ^{13}C NMR (Table I) spectra of **1** showed signals ascribable to three tert.-methyl [δ 0.82 (19-H_3), 0.88 (20-H_3), 1.05 (17-H_3)], a hydroxymethyl [δ 3.08, 3.57 (both d, J=11Hz, 18-H_2)], a trisubstituted olefin [δ 5.31 (s,



trifolione A (**1**) : R=H

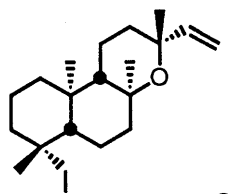
1a : R=Ac



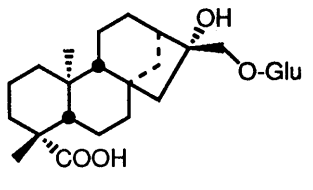
trifolione B (**2**) : R^1=CH_3, R^2=OH

trifolione C (**3**) : R^1=CH_2OH, R^2=H

trifolione D (**4**) : R^1=CH_2OH, R^2=OH

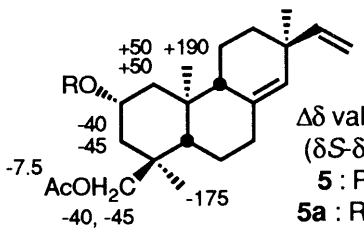


sagittarioside a (**6**)



Glu : β-D-glucopyranosyl

sagittarioside b (**7**)



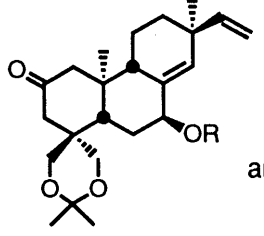
Δδ values in Hz

(δ*S*-δ*R*, 500MHz)

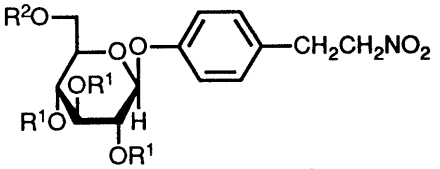
5 : R=H

5a : R=(-)-(*S*)-MTPA

5b : R=(+)-(*R*)-MTPA



4a : R=*p*-Br-benzoyl



arabinothalictoside (**8**) : R^1=H, R^2=α-L-Ara-py.

9 : R^1=R^2=H

9a : R^1=Ac, R^2=H

Table I. The ^{13}C NMR Data for **1**, **1a**, **2**, **3**, **4**, **5**, **6**, and **7**

	1[a]	1a[a]	2[a]	3[b]	4[b]	5[a]	6[b]	7[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.

14-H)] and a vinyl [δ 4.91 (d, J=11Hz), 4.92 (d, J=17Hz)(16-H_2), 5.76 (dd, J=11, 17Hz, 15-H)] groups, which were analyzed completely by use of ^1H-^1H COSY and ^1H-^{13}C COSY. Acetylation of **1** with Ac_2O-pyridine afforded the monoacetate (**1a**),[8] colorless oil, $[\alpha]_D$ -43.8° ($CHCl_3$), $C_{22}H_{32}O_3$, which was treated with $NaBH_4$ in EtOH to furnish **5**,[9] white powder, $[\alpha]_D$ -16.0° ($CHCl_3$), $C_{22}H_{34}O_3$. Comparisons of the ^1H and ^{13}C NMR data for **1**, **1a**, and **5** with those for known diterpenes[10] led us to presume the *ent*-8(14),15-isopimaradiene-18-ol structure of **1**. The connectivities of the quart. carbons (C-2,4,8,10,13) were clarified by COLOC experiment with **1**. Namely, the long-range correlations were observed between the following carbons and protons of **1** (1-C:20-H_3, 2-C:1-H_2[2.23(d, J=13Hz), 2.39(dd, J=2, 13Hz)] & 3-H_2[2.01(dd, J=2, 13Hz), 2.74(d, J=13Hz)], 3-C:19-H_3, 4-C:3-H_2, 8-C:7-H_2, 13-C:14-H). Furthermore, the NOE correlations were observed between the proton pairs of **1** (5-H & 18-H_2 ; 11α-H & 17-H_3 ; 11α-H & 20-H_3 ; 18-H_2 & 19-H_3 ; 19-H_3 & 20-H_3) and **5** (5-H & 18-H_2 ; 11α-H & 17-H_3 ; 11α-H & 20-H_3 ; 5-H & 9-H ; 18-H_2 & 19-H_3 ; 19-H_3 & 20-H_3) in their NOESY spectra. Finally, the CD data for **1** : Δε=-0.31 (290nm)(neg. max), Δε=+1.98(210nm) (pos. max), substantiated the absolute stereostructure of **1**. The absolute stereostructure of trifolione A (**1**) was further confirmed by application of a modified Mosher's method.[11] Thus, the signals due to protons on C-1 and C-20 in the (+)-(*R*)-MTPA ester (**5b**) appeared at higher fields than those of the (-)-(*S*)-MTPA ester (**5a**) (Δδ positive), while the signals due to protons attached to C-3, C-18, and C-19 of **5b** were observed at lower fields as compared to those of **5a** (Δδ negative). Consequently, the absolute configuration at C-2 has been elucidated to be *R* and the absolute structure of **1** has been determined.

Trifolione C (**3**), white powder, $[\alpha]_D$ -13.5°(MeOH), $C_{20}H_{30}O_3$, IR(KBr, cm^{-1}) : 3350, 1700, 1635, 910, 860, CD (EtOH) : Δε=-1.41(290nm)(neg.max), Δε=+2.73(220nm)(pos.max), positive FAB-MS (*m/z*) : 341(M+Na)$^+$, showed signals ascribable to two tert.-methyl, two hydroxymethyl, a trisubstituted olefin and a vinyl functions in its ^1H NMR spectrum.[12] The ^1H and ^{13}C NMR data for **3** were found similar to those data for **1**, except for some signals around the 19-hydroxyl group of **3**. Furthermore, the long-range correlations (1-C:20-H_3, 2-C:1-H_2, 7-C:14-H, 10-C:20-H_3, 12-C:17-H_3, 19-C:5-H) and the NOE correlations (5-H & 9-H ; 5-H & 18-H_2 ; 11α-H & 17-H_3 ; 11α-H & 20-H_3 ; 19-H_2 & 20-H_3) were observed in the COLOC and NOESY spectra of **3**. Finally, the CD data for **3** substantiated its absolute stereostructure as shown.

The structures of trifolione B (**2**)[13] and trifolione D (**4**)[14] have been elucidated in the same way. Based on the ^1H and ^{13}C NMR (Table I) analysis, it was concluded that **2** and **4** have the same skeletal conformation as trifoliones A (**1**) and C (**3**), regardless of the presence of 7β-hydroxyl group. The NOE correlations were observed between the proton pairs of **2** (5-H & 18-H_2, 7-H & 14-H ; 11α-H & 17-H_3 ; 11α-H & 20-H_3) and **4** (5-H & 18-H_2 ; 5-H & 9-H ; 7-H & 14-H ; 11α-H & 17-H_3 ; 11α-H & 20-H_3 ; 19-H_2 & 20-H_3). Comparison of ^1H-^1H coupling constants for **3** and **4** with those for known *ent*-isopimarane type diterpene having 7-hydroxyl group[15] and the CD data of **3** and **4** has led us to formulate their absolute stereostructures **3** and **4**. Furthermore, the absolute configuration of **4** was determined by applying the exciton chirality method[16] to the allylic benzoyl derivative of **4**. Thus the 7-*O*-*p*-bromobenzoate (**4a**), prepared from **4** by introduction of the isopropyridene group with 2,2-dimethoxypropane and *p*-TsOH•H_2O followed by

Table II. Inhibitory Effects of Trifoliones (**1-4**) and Related Diterpenes from *Sagittaria trifolia* 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	0	30.2±14.9
Sclareol	0	0
Ent-kaur-16-en-19-oic acid	0	0
Ent-19-hydroxy-13-epi-manoyl oxide	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.

p-bromobenzoylation, showed a positive Cotton curve[Δε=+4.28(237nm)] to substantiate 7*S* configuration of trifolione D (**4**).

Sagittarioside a (**6**)[17] showed signals ascribable to β-D-glucopyranosyl moiety and *ent*-19-hydroxy-13-epi-manoyl oxide structure in the ^1H and ^{13}C NMR spectra. Based on the above-mentioned evidence and the NOESY experiment, the structure of sagittarioside a (**6**) has been determined. Sagittarioside b (**7**),[18] showed signals due to β-D-glucopyranosyl moiety and 16α,-17-dihydroxy-*ent*-kauran-19-oic acid [5] in its ^1H and ^{13}C NMR spectra. Observation of the glycosidation shift[19] around C-17 position and the NOE correlation between the proton pairs of **7** (1'-H & 17-H_2) led us to formulate the structure of **7** as shown. The structure of arabinothalictoside (**8**, 6'-*O*-α-L-arabinopyranosylthalictoside)[20] was determined by the synthesis from thalictoside (**9**).[21] Thus, monomethoxy-tritylation of **9** followed by acetylation and detritylation yielded 2',3',4-tri-*O*-acetylthalictoside (**9a**) which was subjected to glycosidation with *O*-(2,3,4-tri-*O*-acetyl-L-arabinopyranosyl) trichloroacetimidate in CH_2Cl_2 in the presence of BF_3-etherate to give hexaacetylarabinothalictoside.

Inhibitory effects of trifoliones A-D(**1**~**4**) and related diterpene constituents from *Sagittaria trifolia* on histamine release from rat mast cells induced by compound 48/80 or calcium ionophore A-23187 are summarized in Table II. Among the compounds tested, trifoliones (**1**~**4**) showed more potent inhibitory activity than DSCG and tranilast on histamine release from cells. On the other hand, isoabienol exhibited very little inhibitory effect, and other diterpenes also did not possess inhibitory activity.

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