SAUSSUREAMINES A, B, C, D, AND E, NEW ANTI-ULCER PRINCIPLES FROM CHINESE SAUSSUREAE RADIX

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Five new amino acid-sesquiterpene adducts, saussureamines A, B, C, D, and E, were isolated from Chinese Saussureae Radix, the dried root of *Saussurea lappa* CLARKE, together with a new lignan glycoside, (-)-massoniresinol 4"-*O*-β-D-glucopyranoside. Their structures were determined on the basis of chemical and physicochemical evidence. Among of these compounds, saussureamines A, B, C showed anti-ulcer effect on HCl / ethanol-induced lesions in rats, and saussureamine A also exhibited a inhibitory activity on stress-induced ulcer formation in mice. During the course of these studies, facile conversion from sesquiterpene having an α-methylene γ-lactone function to amino acid-sesquiterpene adduct has been accomplished by means of Michael type addition reaction.

KEYWORDS Saussureae Radix; *Saussurea lappa*; amino acid-sesquiterpene adduct; saussureamine; anti-ulcer activity; Michael type addition

Saussureae Radix (Mokko in Japanese), the dried root of *Saussurea lappa* CLARKE (Compositae), is known as an aromatic stomachic and frequently prescribed in Chinese and Japanese traditional medicine. It has also been used as an important fragrance. Chemical studies on Saussureae Radix originating in India have been carried out by many investigators, and the presence of various sesquiterpenes such as costunolide (**1**) and dehydrocostus lactone (**2**) from the less polar fraction of the Radix has so far been reported.[1] However, no work has been reported on the chemical constituents of Chinese Saussureae Radix, which is now in common use in Japan. As a part of our characterization studies on bioactive constituents of naturally occurring crude drug,[2] we have reported the inhibitory effect of **1** on stress-induced ulcer formation and the gastrointestinal motility-enhancing effect of **2**.[3] In a continuing study, we have isolated five new amino acid-sesquiterpene adducts named saussureamines A (**3**), B (**4**), C (**5**), D (**6**), and E (**7**) from Chinese Saussureae Radix. This paper deals with the structure elucidation of these amino acid-sesquiterpene adducts and their effects against gastric ulcerations. In addition, a new lignan glycoside, (-)-massoniresinol 4"-*O*-β-D-glucopyranoside (**10**), was chemically elucidated.

The MeOH extract of Chinese Saussureae Radix was partitioned into AcOEt and water to furnish the AcOEt soluble portion[4] and the water phase. The water phase was further partitioned with 1-BuOH, and the 1-BuOH soluble portion was subjected to XAD-2, ordinary and reversed phase silica gel, and Sephadex LH-20 column chromatography to afford **3** (0.0011% from the crude drug), **4** (0.0021%), **5** (0.0006%), **6** (0.0001%), **7** (0.0002%) and **10** (0.0009%) together with picriside B (0.0059%),[5] syringin (0.0070%),[5] and (-)-olivil 4"-*O*-β-D-glucopyranoside (0.0004%).[5]

Saussureamine A (**3**), colorless prisms, mp 115~117°C, $[\alpha]_D$ +36.7° (MeOH), $C_{20}H_{29}O_4N$, positive FAB-MS (*m/z*) : 348 $(M+H)^+$, was positive for Dragendorff reagent. The IR spectrum of **3** showed the presence of γ-lactone (1780 cm^{-1}), and carboxyl (3400, 1630 cm^{-1}) functions. The ^1H-[6] and ^{13}C-NMR (Table I) spectra of **3** showed signals ascribable to

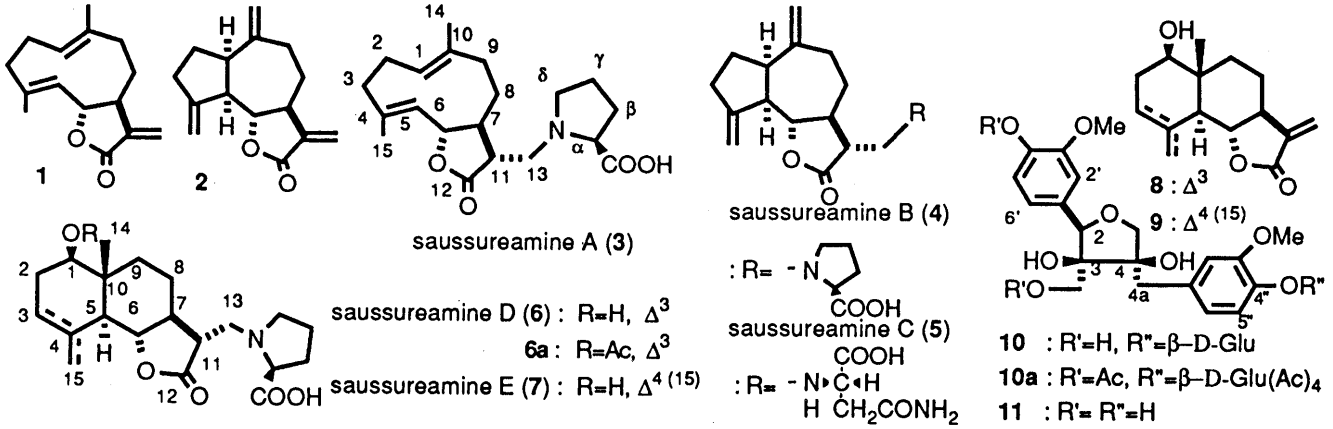

Table I. ^{13}C NMR Data for Saussureamines A-E (**3-7**) (75MHz, pyridine-d_5, δc)

	3	4	5	6	7
C-1	126.7	48.3	44.6	74.4	77.0
C-2	28.5	34.2	37.4	33.7	36.4
C-3	39.5	31.6	32.2	122.4	33.8
C-4	140.0	154.2	150.0	133.7	144.6
C-5	128.5	53.8	51.6	50.1	48.3
C-6	81.5	87.0	85.3	81.5	79.2
C-7	48.7	48.7	46.7	50.9	52.7
C-8	26.3	30.9	29.6	23.3	23.2
C-9	41.1	39.7	38.3	35.5	31.9
C-10	137.5	152.3	152.1	41.2	43.1
C-11	47.7	45.6	46.4	45.7	46.0
C-12	178.1	179.3	177.3	178.3	178.2
C-13	52.0	53.6	46.3	52.5	52.0
C-14	17.1	110.0	108.1	11.4	11.7
C-15	16.0	112.6	110.9	23.7	109.1
C-α	67.7	68.9	59.5	67.5	67.0
C-β	29.5	34.1	32.0	29.6	29.3
C-γ	24.2	25.6	173.5	24.1	23.8
C-δ	54.5	55.7	-	54.1	53.8
C=O	176.6	177.9	175.1	176.7	176.2

Table II. Effect of Saussureamines A-E (**3-7**) and Cetraxate on HCl / Ethanol Induced Gastric Ulcer in Rats

Treatment	Dose (mg / kg)	N	Total length (mm) (Mean ± S.E.)	Inhibition (%)
Control	-	8	72.4±9.0	-
Saussureamine A (**3**)	50	5	42.3±8.3*	41.6
	100	6	29.1±16.6*	59.8
Saussureamine B (**4**)	50	6	9.3±8.6**	87.2
	100	4	9.4±3.9**	87.1
Saussureamine C (**5**)	50	6	57.3±16.3	20.9
	100	6	23.1±5.5**	68.1
Saussureamine D (**6**)	100	5	63.8±13.9	11.9
Saussureamine E (**7**)	100	5	63.7±16.9	12.0
Cetraxate	300	6	9.3±8.6**	87.2

*$p<0.05$, **$p<0.01$.

structure of saussureamine A was shown to be **3**. Finally, treatment of **1** with L-proline in EtOH in the presence of Et_3N (90°C, 1h) furnished **3** in 76% yield. Thus, it was inferred that the Michael type addition reaction for the α-methylene γ-lactone part in **1** with L-proline proceeded stereoselectively to provide thermodynamically favored addition product **3** having the α-configurated L-proline side chain.[7]

Saussureamine B (**4**), white powder, $[\alpha]_D$ -25.9° (MeOH), $C_{20}H_{27}O_4N$, IR (KBr, cm^{-1}): 3400, 1770, 1640, positive FAB-MS (*m/z*) : 346 $(M+H)^+$, was positive for Dragendorff reagent and liberated dehydrocostus lactone (**2**) and L-proline by acid treatment. The Michael type addition reaction of **2** with L-proline under the same conditions as for **1** furnished **4** (70%). Based on ^1H-[8] and ^{13}C-NMR (Table I) examinations for **4** and observation of the NOE correlation between the 11β-H and 6β-H, the structure of saussureamine B (**4**) was determined. [9]

Saussureamine C (**5**), white powder, $[\alpha]_D$ -17.2 ° (MeOH), $C_{19}H_{26}O_5N_2$, IR (KBr, cm^{-1}): 3200, 1760, 1675, 1640, positive FAB-MS (*m/z*) : 363 $(M+H)^+$ was positive for Dragendorff reagent and in Ninhidrin test. Treatment of **2** with L-asparagine in 70% aq. EtOH in the presence of Et_3N (90°C, 2h) provided **5** (48%). Comparisons in detail of ^1H-[10] and ^{13}C-NMR (Table I) data for **5** with those for **2** and L-asparagine and NOE observation between the 11β-H and 6β-H in **5** led us to confirm the structure of saussureamine C (**5**) as shown.

Saussureamine D (**6**), colorless needles, mp 235~237°C (MeOH-H_2O), $[\alpha]_D$ +13.3 ° (MeOH), $C_{20}H_{29}O_5N$, IR (KBr, cm^{-1}): 3200, 1765, 1634, positive FAB-MS (*m/z*) : 370 $(M+Li)^+$, negative FAB-MS (*m/z*): 362 $(M-H)^-$, positive for Dragendorff reagent, had a tertiary methyl, a vinyl methyl, a secondary hydroxyl and a trisubstituted olefin group together with γ-lactone and L-proline moiety as indicated by its ^1H-[11] and ^{13}C-NMR (Table I) data. Ordinary acetylation of **6** furnished the monoacetate (**6a**).[12] Detailed comparisons of ^1H- and ^{13}C-NMR data for **6** with those for **3**, **4** and known eudesmanolides suggested the structure **6** linked with L-proline at 13-position in santamarin (**8**) which was isolated from *Chrysantemum parthenium* .[13] Furthermore, NOE correlations were observed between the proton pairs of **6** [1α-H / 5α-H; 14β-H_3 / 6β-H; 6β-H / 11β-H]. Finally, treatment of **8** with L-proline in EtOH in the presence of Et_3N provided **6** in 91% yield. Thus, the structure of saussureamne D (**6**) was substantiated.

The ^1H-[14] and ^{13}C-NMR (Table I) spectra of saussureamine E (**7**), colorless needles, mp 150~153°C (MeOH-H_2O), $[\alpha]_D$ + 33.7° (MeOH), $C_{20}H_{29}O_5N$, IR (KBr, cm^{-1}): 3200, 1755, 1630, positive FAB-MS (*m/z*) : 364 $(M+H)^+$, positive for Dragendorff reagent, were closely similar to those of **6** except for some signals due to the olefin moiety. Treatment of reynosin (**9**), which was isolated from *Ambrosia confertiflora*[15] with L-proline under the same conditions as for **8**, furnished **7** (82%). Based on this evidence and NOE observation between the 6β-H and 11β-H, the structure of saussureamine E (**7**) was clarified.

(-)-Massoniresinol 4"-*O*-β-D-glucopyranoside (**10**), white powder, $[\alpha]_D$ - 67.0° (MeOH), $C_{26}H_{34}O_{13}$, UV [MeOH, nm (ε)] : 228 (8500), 279 (4030), IR (KBr, cm^{-1}): 3200, 1600, 1510, positive FAB-MS (*m/z*) : 577 $(M+Na)^+$, furnished the hexaacetate (**10a**), white powder, $[\alpha]_D$ - 27.5° (MeOH), $C_{38}H_{46}O_{19}$, by ordinary acetylation. Methanolysis of **10** with 9%

HCl-MeOH liberated (-)-massoniresinol (**11**)[16] and methyl D-glucopyranoside. The ^{1}H- and ^{13}C-NMR signals[17] of **10** could be analyzed completely by use of ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY and COLOC experiments. The NOEs were observed between the following pairs of protons in **10** : anomeric-H / 5"-H ; 6"-H / 4a-H$_2$; 2-H / 6'-H. Based on this evidence and comparison of ^{13}C-NMR data for **10** with those for **11**, the structure **10** was determined as shown.

Table III. Effect of Saussureamines A-E (**3**-**7**), Costunolide (**1**), and Dehydrocostus lactone (**2**) and Cimetidine on Stress-induced Gastric Ulcer in Mice

Treatment	Dose (mg / kg)	N	Total length (mm) (Mean ± S.E.)	Inhibition (%)
Control	-	7	3.00±0.00	-
Saussureamine A (**3**)	100	7	1.71±0.18**	43.0
	200	7	1.29±0.42**	57.0
Saussureamine B (**4**)	200	9	2.33±0.29	22.3
Saussureamine C (**5**)	200	9	2.78±0.15	7.3
Saussureamine D (**6**)	200	10	2.30±0.30	23.3
Saussureamine E (**7**)	200	10	2.60±0.16	13.3
Costunolide (**1**)	200	9	2.56±0.24	14.7
Dehydrocostus lactone (**2**)	200	9	2.33±0.24	22.3
Cimetidine	150	10	0.90±0.31**	70.0

**p<0.01

The inhibitory activities of saussureamines A (**3**), B (**4**), C (**5**), D (**6**), and E (**7**) on HCl / ethanol-induced gastric lesions in rats and on the formation of gastric ulcer in restrained and water-immersed mice are summarized in Table II and III, respectively. Among compounds tested for their effect on HCl / ethanol-induced gastric lesions, **4** showed a more potent anti-ulcer effect than cetraxate, and the inhibitory effect of **3** and **5** was comparable to cetraxate. As given in Table III, **3** showed potent inhibitory effect on stress-induced gastric ulcer formation, while **4** and **6** were found to exhibit a weak inhibitory activity.

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