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# Absolute Stereostructures and Syntheses of Saussureamines A, B, C, D and E, Amino Acid–Sesquiterpene Conjugates with Gastroprotective Effect, from the Roots of *Saussurea lappa*

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**Abstract**—From the methanolic extract of the dried roots of *Saussurea lappa* Clarke, Saussureae Radix, five amino acid–sesquiterpene conjugates, saussureamines A, B, C, D and E, were isolated together with a lignan glycoside, (−)-massoniresinol 4″-*O*-β-D-glucopyranoside. Their stereostructures were determined on the basis of chemical and physicochemical evidence. In addition, saussureamines and the related amino acid–sesquiterpene conjugates were synthesized using a Michael type addition reaction of amino acid to the α-methylene-γ-lactone moiety of sesquiterpenes. Saussureamines A, B and C, costunolide and dehydrocostus lactone showed a gastroprotective effect on acidified ethanol-induced gastric mucosal lesions in rats. Saussureamines A also exhibited an inhibitory effect on gastric mucosal lesions induced by water-immersion stress in mice. 

## Introduction

The dried roots of *Saussurea* (*S.*) *lappa* Clarke (Compositae) have been used as a Chinese herbal medicine, Saussureae Radix, which is listed in the Japanese Pharmacopoeia XIII as an aromatic stomachic, and also have been used as an important fragrance. As chemical constituents of this plant, several sesquiterpenes, such as costunolide (**7**) and dehydrocostus lactone (**8**), have been isolated from Indian *S. lappa*.[1–3] In the course of our studies on bioactive constituents of Chinese natural medicines,[4–7] we found that the methanolic extract of Chinese *S. lappa* showed a potent inhibitory effect on acidified ethanol-induced gastric lesions in rats. From the methanolic extract of Chinese *S. lappa*, we isolated five new amino acid–sesquiterpene conjugates named saussureamines A (**1**), B (**2**), C (**3**), D (**4**) and E (**5**) and a new lignan glycoside, (−)-massoniresinol 4″-*O*-β-D-glucopyranoside (**6**). This paper presents a full account of the structure elucidation of these saussureamines (**1**–**5**) and a lignan glycoside (**6**) as well as the syntheses of saussureamines (**1**–**5**) and the related amino acid–sesquiterpene conjugates (**11**–**24**) from costunolide (**7**) and dehydrocostus lactone (**8**), which are the principal sesquiterpene constituents in *S. lappa*. In addition, we describe their effects on gastric lesions induced by acidified ethanol or water-immersion stress.[8]

## Results and Discussion

The isolation of the chemical constituents from *S. lappa* was carried out through the following procedure. The dried roots of Chinese *S. lappa* were extracted with methanol under reflux. The methanolic extract was partitioned into an ethyl acetate and water mixture to give an ethyl acetate-soluble portion and an aqueous phase. The aqueous phase was further extracted with 1-butanol to give a 1-butanol-soluble portion and a water-soluble portion. The ethyl acetate-soluble portion was subjected to silica gel column chromatography to furnish principal sesquiterpene constituents costunolide (**7**,[1] 1.07% from the natural medicine) and dehydrocostus lactone (**8**,[9] 1.43%). Furthermore, the other fraction was subjected to recycling HPLC to furnish α- and β-costols mixture[10–12] (0.005%) and (−)-elema-1,3,11(13)-trien-12-ol[12] (0.002%). The 1-butanol-soluble portion was subjected to Amberlite XAD-2, silica gel, reversed-phase silica gel, and finally Sephadex LH-20 column chromatography to furnish saussureamines A (**1**, 0.0011%), B (**2**, 0.0021%), C (**3**, 0.0006%), D (**4**, 0.0001%) and E (**5**, 0.0002%), (−)-massoniresinol 4″-*O*-β-D-glucopyranoside (**6**, 0.0009%), picriside B[13] (0.0059%), syringin[14] (0.0070%), and (−)-olivil 4″-*O*-β-D-glucopyranoside[15] (0.0004%) (Chart 1).

### Saussureamines A (1), B (2) and C (3)

Saussureamine A (**1**) was isolated as colorless prisms of mp 115–117°C with positive optical rotation ($[\alpha]_D^{20}$=+36.7°) and was deduced to possess a nitrogen function based on TLC examination using the Dragendorff reagent. The

*Keywords*: amino acids and derivatives; Michael reactions; pharmacologically active compounds; terpenes and terpenoids.

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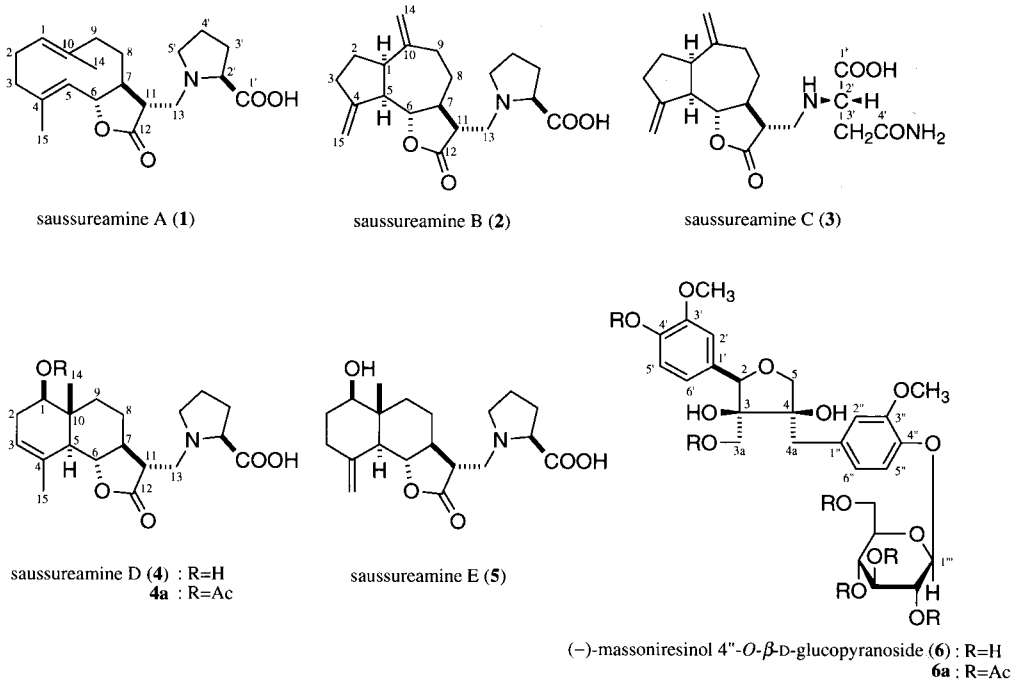



**Chart 1.**

positive-ion fast atom bombardment (FAB)-MS of **1** showed a quasimolecular ion peak at *m/z* 348 $(M+H)^+$, and high-resolution MS analysis revealed the molecular formula of **1** to be $C_{20}H_{29}NO_4$. The IR spectrum of 1 showed absorption bands at 3400, 1780 and 1630 $cm^{-1}$ ascribable to carboxylate and γ-lactone functions. The $^1H$ NMR (pyridine-$d_5$) and $^{13}C$ NMR (Table 1) spectra of **1** showed signals assignable to two tertiary methyl [δ 1.34, 1.60 (both s, 14 and 15-$H_3$)], a methylene with a characteristic ABX type coupling pattern [δ 3.22 (dd, *J*=3, 13 Hz), 3.41 (dd, *J*=6, 13 Hz), 13-$H_2$], a methine bearing the oxygen function [δ 4.66 (dd, *J*=9, 10 Hz, 6-H)], and two olefins [δ 4.70 (m, 1-H), 4.80 (d, *J*=10 Hz, 5-H)] together with seven methylene groups and three methine groups. The proton and carbon

**Table 1.** $^{13}C$ NMR data for saussureamines A (**1**), B (**2**), C (**3**), D (**4**), D monoacetate (**4a**) and E (**5**) and amino acid conjugates (**16**–**24**)

| | **1**[a] | **2**[a] | **3**[a] | **4**[a] | **4a**[a] | **5**[a] | **16**[b] | **17**[c] | **18**[b] | **19**[c] | **20**[b] | **21**[b] | **22**[b] | **23**[b] | **24**[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C-1 | 126.7 | 47.1 | 46.8 | 74.4 | 77.0 | 77.4 | 126.9 | 126.2 | 46.9 | 46.2 | 47.2 | 47.3 | 47.2 | 47.2 | 47.3 |
| C-2 | 28.5 | 32.9 | 37.8 | 33.7 | 29.8 | 36.8 | 26.4 | 25.7 | 30.2 | 29.4 | 30.3 | 30.3 | 30.3 | 30.3 | 30.3 |
| C-3 | 39.5 | 30.3 | 32.6 | 122.4 | 124.2 | 34.2 | 39.6 | 38.8 | 32.8 | 32.0 | 32.8 | 32.8 | 32.8 | 32.8 | 32.8 |
| C-4 | 140.0 | 152.8 | 150.5 | 133.7 | 134.0 | 144.9 | 139.9 | 139.6 | 152.8 | 152.3 | 152.7 | 152.7 | 152.7 | 152.7 | 152.7 |
| C-5 | 128.5 | 52.4 | 52.0 | 50.1 | 49.5 | 48.7 | 128.4 | 127.4 | 52.1 | 51.5 | 52.4 | 52.5 | 52.4 | 52.3 | 52.4 |
| C-6 | 81.5 | 85.7 | 85.7 | 81.5 | 80.8 | 79.6 | 81.7 | 80.4 | 86.0 | 84.3 | 85.4 | 85.4 | 85.4 | 85.5 | 85.5 |
| C-7 | 48.7 | 44.6 | 45.0 | 50.9 | 50.5 | 53.0 | 50.1 | 49.7 | 45.3 | 45.3 | 45.2 | 45.3 | 45.3 | 45.2 | 45.4 |
| C-8 | 26.3 | 29.6 | 30.0 | 23.3 | 23.0 | 23.5 | 28.6 | 27.2 | 32.6 | 31.4 | 32.8 | 32.8 | 32.8 | 32.7 | 32.8 |
| C-9 | 41.1 | 38.2 | 38.7 | 35.5 | 34.7 | 32.2 | 41.2 | 40.4 | 38.0 | 36.8 | 37.9 | 37.8 | 38.0 | 38.0 | 37.8 |
| C-10 | 137.5 | 150.9 | 152.5 | 41.2 | 39.7 | 43.4 | 137.4 | 136.7 | 150.8 | 150.2 | 150.7 | 150.7 | 150.7 | 150.7 | 150.7 |
| C-11 | 47.7 | 47.2 | 47.1 | 45.7 | 46.0 | 46.3 | 48.3 | 47.1 | 47.3 | 46.5 | 48.3 | 48.5 | 48.3 | 48.5 | 48.4 |
| C-12 | 178.1 | 177.9 | 177.7 | 178.3 | 178.3 | 178.6 | 178.5 | 176.3 | 178.5 | 176.3 | 177.6 | 177.7 | 177.7 | 177.8 | 177.9 |
| C-13 | 52.0 | 52.4 | 46.8 | 52.5 | 52.3 | 52.4 | 46.9 | 29.8 | 46.9 | 30.3 | 48.0 | 46.9 | 46.9 | 46.8 | 47.0 |
| C-14 | 17.1 | 108.7 | 108.5 | 11.4 | 12.3 | 12.0 | 16.1 | 15.8 | 111.4 | 111.2 | 111.5 | 111.6 | 111.5 | 111.5 | 111.6 |
| C-15 | 16.0 | 111.4 | 111.3 | 23.7 | 23.5 | 109.5 | 17.1 | 16.8 | 108.6 | 107.9 | 108.8 | 108.9 | 108.8 | 108.8 | 108.8 |
| *C*$H_3$CO– | | | | | 20.9 | | | | | | | | | | |
| $CH_3$CO– | | | | | 170.3 | | | | | | | | | | |
| C-1′ | 176.6 | 176.3 | 175.5 | 176.7 | 176.7 | 176.5 | 180.8 | 168.6 | 179.9 | 168.4 | 176.3 | 178.1 | 176.9 | 176.1 | 177.4 |
| C-2′ | 67.7 | 67.7 | 59.9 | 67.5 | 67.6 | 67.3 | 64.5 | 53.9 | 64.5 | 53.8 | 52.3 | 61.6 | 64.3 | 65.4 | 62.0 |
| C-3′ | 29.5 | 32.7 | 32.5 | 29.6 | 29.8 | 29.6 | 31.1 | 35.0 | 30.6 | 34.9 | | 43.3 | 40.1 | 64.1 | 33.7 |
| C-4′ | 24.2 | 24.3 | 173.9 | 24.1 | 24.3 | 24.1 | 26.7 | | 26.1 | | | 25.9 | 139.5 | | 31.2 |
| C-5′ | 54.5 | 54.4 | | 54.1 | 54.5 | 54.1 | 41.4 | | 41.6 | | | 22.4[d] | 130.0 | | 15.2 |
| C-6′ | | | | | | | 159.0 | | 158.4 | | | 23.2[d] | 128.6 | | |
| C-7′ | | | | | | | | | | | | | 126.6 | | |

[a] Measured in pyridine-$d_5$ at 75 MHz.
[b] Measured in pyridine-$d_5$ at, 125 MHz.
[c] Measured in DMSO-$d_6$ at 125 MHz.
[d] May be interchangeable within the same column.

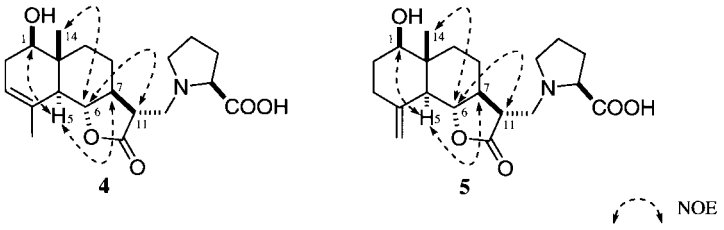



**Figure 1.** NOE correlations of **4** and **5**.

signals of **1** were shown to be very similar to those of costunolide (**7**), except for the 13-*exo*-methylene position of **7**. Treatment of **1** with 1% aqueous hydrochloric acid (HCl) at room temperature liberated **7** and L-proline. This evidence led us to confirm the structure of **1**, in which L-proline bonded at the 13-position of **7**.

In the nuclear Overhauser effect (NOE) experiment on **1**, an NOE correlation was observed between the 6β-proton and the 11β-proton [$\delta$ 2.56 (m)] signals and the stereostructure of the 11-position in **1** was clarified to be an α-configuration. On the basis of this evidence, the absolute stereostructure of saussureamine A (**1**) was determined.

Saussureamine B (**2**), isolated as a white powder with negative optical rotation ($[\alpha]_D^{20}=-25.9°$), was also deduced to possess a nitrogen function based on the Dragendorff test. The molecular formula $C_{20}H_{27}NO_4$ of **2** has been determined from the quasimolecular ion peak at *m/z* 346 $(M+H)^+$ in the positive-ion FAB-MS of **2** and by high-resolution MS measurement. The IR spectrum of **2** showed absorption bands ascribable to carboxylate (3400, 1640 $cm^{-1}$) and γ-lactone (1770 $cm^{-1}$) functions. The $^1H$ NMR (pyridine-$d_5$) and $^{13}C$ NMR (Table 1) spectra of **2** showed signals assignable to an ABX type methylene [$\delta$ 3.14 (dd, *J*=4, 13 Hz), 3.38 (dd, *J*=5, 13 Hz), 13-$H_2$], a methine bearing a oxygen function [$\delta$ 4.00 (dd, *J*=9, 9 Hz, 6-H)], and two *exo*-methylene [$\delta$ 4.70, 4.80 (both br s, 14-$H_2$), 5.07, 5.33 (both br s, 15-$H_2$)] together with four methylene groups, four methine groups, and a L-proline moiety. The proton and carbon signals in the $^1H$ and $^{13}C$ NMR spectra of **2** were shown to be very similar to those of dehydrocostus lactone (**8**), except for the 13-*exo*-methylene position of **8**. In addition, **2** was liberated **8** and L-proline by 1% aqueous HCl treatment, and the NOE experiment on **2** showed an NOE correlation between the 6β-proton and the 11β-proton [$\delta$ 2.85 (m)] signals. Consequently, the stereostructure of saussureamine B (**2**) was determined.

Saussureamine C (**3**) was also isolated as a white powder with negative optical rotation ($[\alpha]_D^{20}=-17.2°$) and was positive in the Dragendorff and Ninhydrin tests. The positive-ion FAB-MS of **3** showed a quasimolecular ion peak at *m/z* 363 $(M+H)^+$, and high-resolution MS analysis revealed the molecular formula of **3** to be $C_{19}H_{26}N_2O_5$. In the IR spectrum of **3**, absorption bands due to carboxylate, γ-lactone, and amide functions were observed at 3200, 1760, 1675 and 1640 $cm^{-1}$. The $^1H$ NMR (pyridine-$d_5$) and $^{13}C$ NMR (Table 1) spectra of **3** showed signals assignable to a dehydrocostus lactone moiety, an ABX type methylene [$\delta$ 3.23 (m), 3.45 (dd, *J*=6, 13 Hz), 13-$H_2$], a methine bearing the oxygen function [$\delta$ 3.96 (dd, *J*=9, 9 Hz, 6-H)], two *exo*-methylene [$\delta$ 4.70, 4.82 (both br s, 14-$H_2$), 5.06, 5.31 (both br s, 15-$H_2$)], and an L-asparagine moiety. Acid treatment of **3** furnished **8** and L-asparagine, and the 11-stereostructure of **3** was clarified by an NOE experiment, which showed NOE correlation between the 6β-proton and the 11β-proton [$\delta$ 2.67 (m)]. Consequently, the stereostructure of saussureamine C (**3**) was determined as shown.

## Saussureamines D (4) and E (5)

Saussureamine D (**4**) was isolated as colorless needles of mp 235–237°C (MeOH–$H_2O$) with positive optical rotation ($[\alpha]_D^{20}=+13.3°$) and the Dragendorff test for **4** was positive. The positive- and negative-ion FAB-MS of **4** showed a quasimolecular ion peak *m/z* 370 $(M+Li)^+$, and 362 $(M-H)^-$. The high-resolution MS analysis revealed the molecular formula of **4** to be $C_{20}H_{29}NO_5$, and the IR spectrum showed absorption bands ascribable to carboxylate (3200, 1634 $cm^{-1}$), and γ-lactone (1765 $cm^{-1}$) functions. The $^1H$ NMR (pyridine-$d_5$) and $^{13}C$ NMR (Table 1) spectra of **4** showed signals assignable to a tertiary methyl [$\delta$ 1.07 (s, 14-$H_3$)], a vinyl methyl [$\delta$ 1.94 (s, 15-$H_3$)], a secondary hydroxyl [$\delta$ 3.81 (dd, *J*=6, 10 Hz, 1-H)], and an olefin [$\delta$ 5.36 (br s, 3-H)] together with γ-lactone and L-proline moiety. The sesquiterpene moiety of **4** have been included a hydroxyl group, since ordinary acetylation of **4** furnished the monoacetate **4a**, of which the $^1H$ NMR (pyridine-$d_5$) and $^{13}C$ NMR (Table 1) spectra showed signals assignable to a acetyl group [$\delta$ 2.04 (s), $\delta$c 20.9, 170.3]. Next, acid treatment of **4** with 1% aqueous HCl at room temperature liberated a known eudesmanolide, santamarine (**9**),[16] and L-proline. Furthermore, NOE correlations were observed between the following protons [1α-H and 5α-H: δ 2.10 (m); 5α-H and 7α-H: δ 2.35 (m); 14-$H_3$ and 6β-H: δ 4.07 (dd, *J*=10, 10 Hz); 6β-H and 11β-H: δ 2.70 (m)] as shown in Fig. 1. Finally, by comparison of the $^1H$ and $^{13}C$ NMR data for **4** with those for **1**, **2**, **9** and L-proline, the stereostructure of saussureamine D (**4**) was established as shown.

Saussureamine E (**5**), also isolated as colorless needles of mp 150–153°C (MeOH–$H_2O$) with positive optical rotation ($[\alpha]_D^{20}=+33.7°$), was positive in the Dragendorff test. The molecular formula $C_{20}H_{29}NO_5$ of **5** has been determined for the quasimolecular ion peak at *m/z* 364 $(M+H)^+$ in the positive-ion FAB-MS and by high-resolution MS measurement. The IR spectrum of **5** showed absorption bands ascribable to carboxylate (3200, 1630 $cm^{-1}$) and γ-lactone (1755 $cm^{-1}$) functions. The $^1H$ NMR (pyridine-$d_5$) and $^{13}C$ NMR (Table 1) spectra of **5** showed signals assignable to a tertiary methyl [$\delta$ 1.03 (s, 14-$H_3$)], a secondary hydroxyl [$\delta$ 3.65 (m, 1-H)], and an *exo*-methylene [$\delta$ 4.99, 5.03 (both br s, 15-$H_2$)] together with γ-lactone and L-proline moiety,

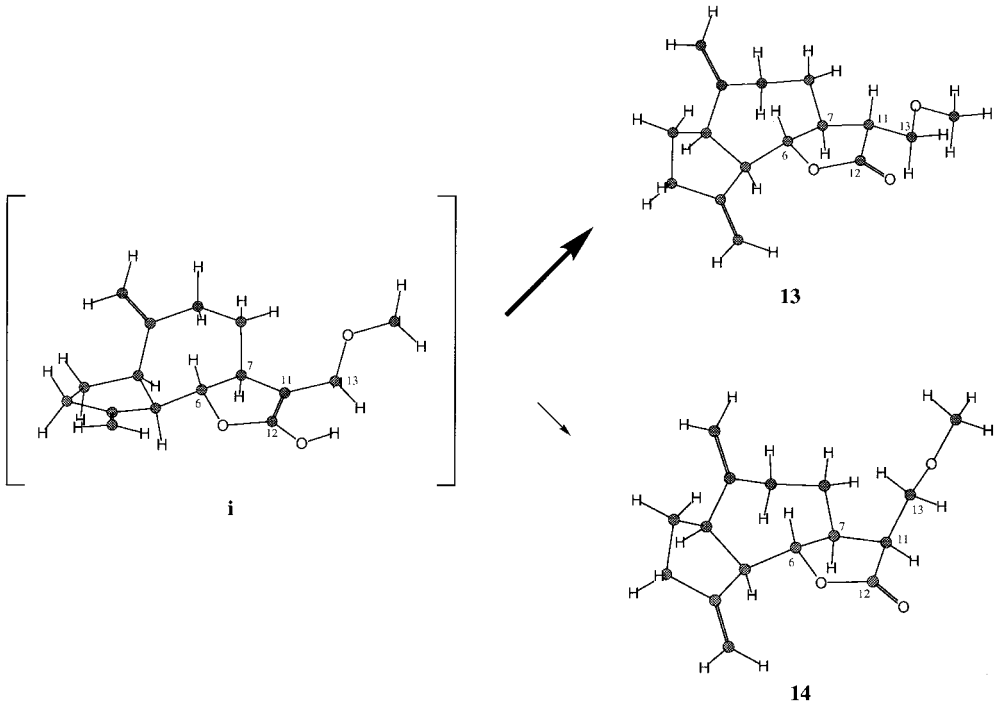



**Figure 2.** Conformations of **13** and **14**.

which were shown to be closely similar to the signals of **4**, except for the 13-*exo*-methylene position of **5**. In addition, treatment of **5** with 1% aqueous HCl furnished reynosin (**10**)[17] and L-proline. Finally, the NOEs were observed between the following proton signals [1α-H and 5α-H: δ 2.10 (m); 5α-H and 7α-H: δ 2.13 (m); 14-$H_3$ and 6β-H: δ 4.22 (dd, *J*=10, 10 Hz); 6β-H and 11β-H: δ 2.78 (ddd, *J*=5, 5, 12 Hz)] as shown in Fig. 1. Consequently, the stereostructure of saussureamine E (**5**) was determined.

**Table 2.** $^{13}C$ NMR data for **11**–**15** (measured in $CDCl_3$ at 125 MHz)

| | **11** | **12** | **13** | **14** | **15** |
|---|---|---|---|---|---|
| C-1 | 47.1 | 47.1 | 46.8 | 47.3 | 127.2 |
| C-2 | 30.1 | 30.2 | 30.0 | 30.2 | 26.1 |
| C-3 | 32.5 | 32.5 | 32.4 | 32.5 | 39.5 |
| C-4 | 151.7 | 151.6 | 151.6 | 151.7 | 140.9 |
| C-5 | 52.1 | 51.8 | 51.6 | 52.6 | 127.0 |
| C-6 | 85.3 | 85.5 | 85.3 | 87.1 | 81.7 |
| C-7 | 47.1 | 46.1 | 43.9 | 43.7 | 50.4 |
| C-8 | 33.0 | 32.6 | 32.4 | 27.7 | 28.4 |
| C-9 | 37.6 | 37.4 | 37.6 | 37.2 | 41.0 |
| C-10 | 150.1 | 149.7 | 149.8 | 149.9 | 136.7 |
| C-11 | 45.8 | 47.7 | 47.8 | 45.8 | 48.2 |
| C-12 | 177.9 | 176.8 | 175.9 | 178.0 | 176.8 |
| C-13 | 52.9 | 29.9 | 68.8 | 69.8 | 29.3 |
| C-14 | 111.5 | 112.0 | 111.5 | 111.8 | 16.1 |
| C-15 | 109.1 | 109.2 | 108.9 | 109.3 | 17.2 |
| | | | | | |
| $-SCH_2CH_2OH$ | | 36.3 | | | 36.4 |
| $-SCH_2CH_2OH$ | | 60.9 | | | 61.0 |
| $-N(CH_2CH_3)_2$ | 47.1 | | | | |
| $-N(CH_2CH_3)_2$ | 11.7 | | | | |
| $-OCH_3$ | | | 59.1 | 59.0 | |

## Syntheses of saussureamines and the related amino acid–sesquiterpene conjugates

We carried out the synthesis of saussureamines A (**1**), B (**2**) and C (**3**) and their related compound (**11**–**24**) from costunolide (**7**) and dehydrocostus lactone (**8**) using a Michael type addition reaction. In order to examine the stereoselectivity and reactivity of the Michael type addition reaction of amino acid for the α-methylene-γ-lactone moiety of sesquiterpene, **8** was treated with several nucleophiles related to amino acid. Namely, treatment of **8** with diethylamine or 2-mercaptoethanol was found to furnish the 11α-addition product (**11** or **12**). By treatment of **8** with sodium methoxide in methanol, the 11α-derivative (**13**) was also obtained as a principal product (88% yield) together with the minor 11β-derivative (**14**, 5%) with a hindered conformation via the enol-form intermediate (**i**) as shown in Fig. 2.[18] Treatment of **8** with sodium acetate or acetic acid in the presence of triethylamine ($Et_3N$) gave no product. Furthermore, treatment of **7** with a mixture of diethylamine and 2-mercaptoethanol yielded an 11α-thioether (**15**). The structures of these synthetic adducts were determined by detail examination of the $^1H$ NMR and $^{13}C$ NMR (Table 2) spectra and the NOE observation between the 11-proton and the 6-proton (**11**–**13**, **15**) or between the 11-proton and the 7-proton (**14**) (Chart 2).

After above mentioned preliminary experiment, saussureamines A (**1**), B (**2**) and C (**3**) were synthesized from amino acids and **7** or **8**. Treatment of **7** and **8** with L-proline in ethanol in the presence of $Et_3N$ was found to selectively furnish **1** and **2** in 76% and 70% yields, respectively. On the other hand, the conjugated addition reaction of L-asparagine to **8** also provided **3** in a 48% yield. These results revealed that the Michael type addition for α-methylene-γ-lactone part in **7** or **8** with L-proline or L-asparagine

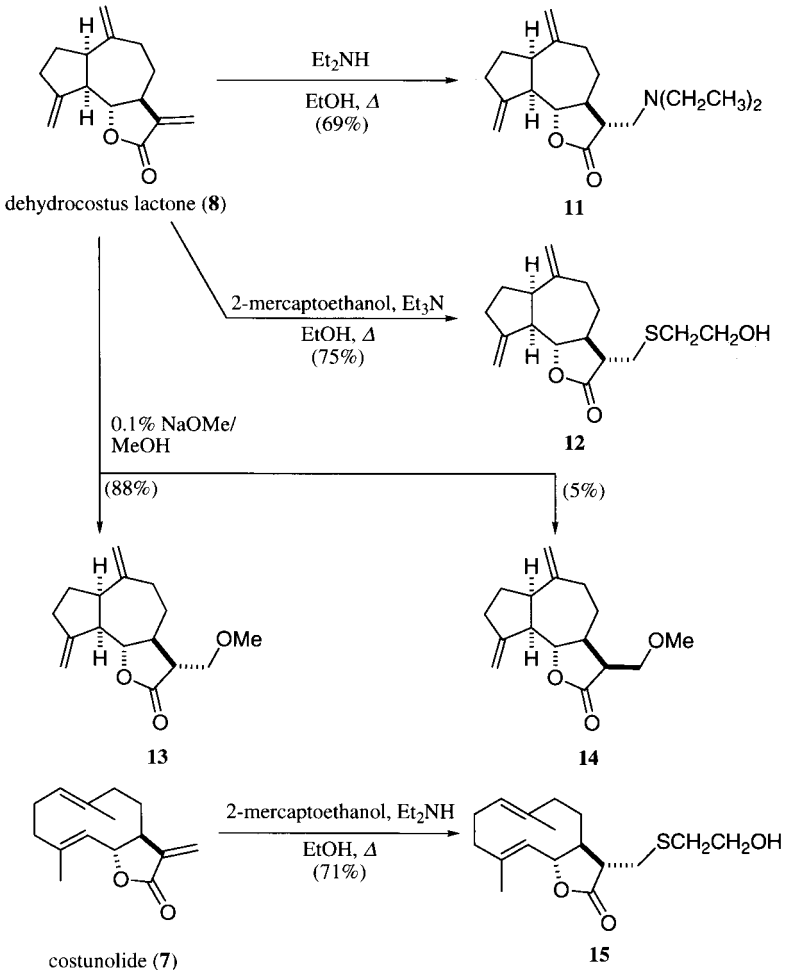
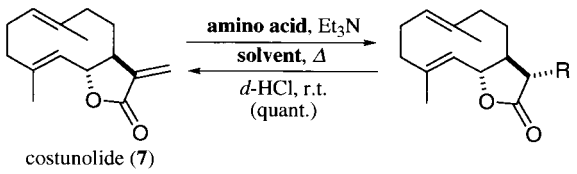
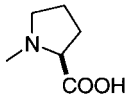
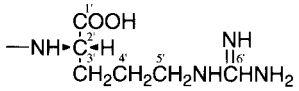
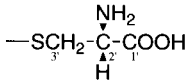


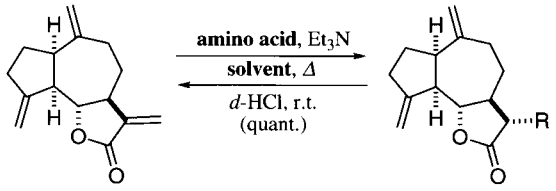

**Chart 2.** Synthesis of **11–15**.



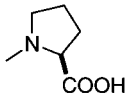
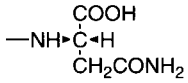
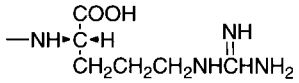
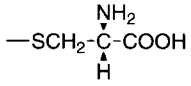
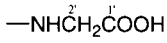
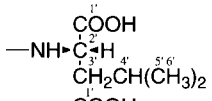
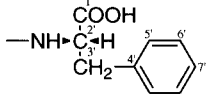
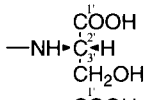
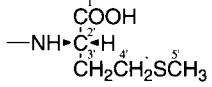

| compound | R | amino acid | reaction condition: solvent | time | yield (%) |
|---|---|---|---|---|---|
| **1** |  | L-proline | EtOH | 1 h | 76 |
| **16** |  | L-arginine | 70%aq. EtOH | 12 h | *61 |
| **17** |  | L-cysteine | 70%aq. EtOH | 1 h | *82 |

*conversion yield

**Chart 3.** Syntheses of amino acid–costunolide conjugates.




dehydrocostus lactone (**8**)

| compound | R | amino acid | reaction condition: solvent | time | yield (%) |
|---|---|---|---|---|---|
| **2** |  | L-proline | EtOH | 1 h | 70 |
| **3** |  | L-asparagine | 70%aq. EtOH | 2 h | 48 |
| **18** |  | L-arginine | 70%aq. EtOH | 12 h | *59 |
| **19** |  | L-cysteine | 70%aq. EtOH | 1 h | 72 |
| **20** |  | glycine | EtOH | 2 h | 83 |
| **21** |  | L-leucine | EtOH | 2 h | 89 |
| **22** |  | L-phenylalanine | EtOH | 7 h | 75 |
| **23** |  | L-serine | EtOH | 5 h | 76 |
| **24** |  | L-methionine | EtOH | 10 h | *54 |

*conversion yield

**Chart 4.** Syntheses of amino acid–dehydrocostus lactone conjugates.

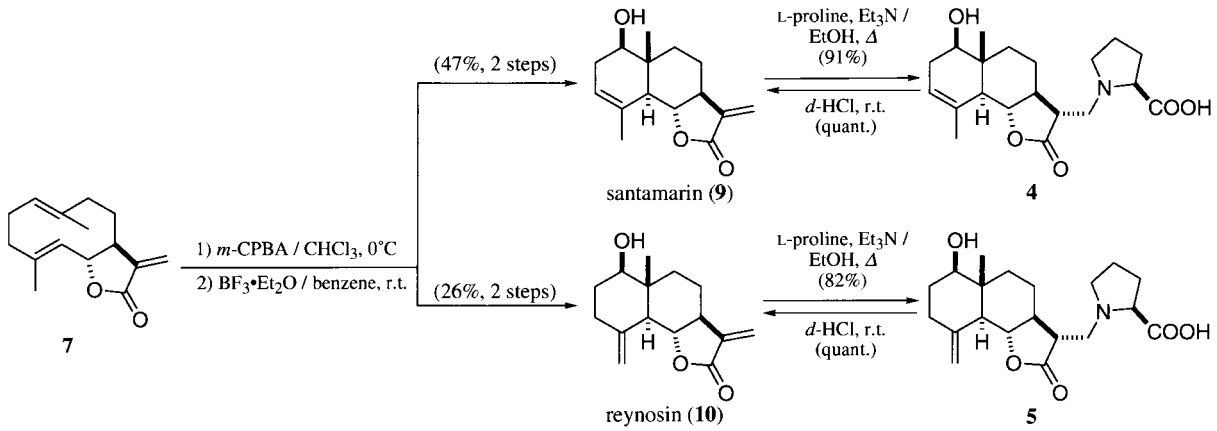



**Chart 5.** Syntheses of **4** and **5**.

**Table 3.** $^{13}C$ NMR data for (−)-massoniresinol 4″-*O*-β-D-glucopyranoside (**6**) and its hexaacetate (**6a**) (measured in $CD_3OD$ at 75 MHz)

| | **6** | **6a** | | **6a** |
|---|---|---|---|---|
| C-2 | 86.1 | 86.4 | $CH_3CO$– | 21.0 |
| C-3 | 82.1 | 82.8 | | 21.1 |
| C-3a | 64.4 | 66.8 | | 21.2 |
| C-4 | 82.3 | 81.5 | | 21.2 |
| C-4a | 40.2 | 40.7 | | 21.3 |
| C-5 | 74.9 | 75.1 | | 21.3 |
| C-1′ | 131.1 | 139.2 | $CH_3CO$– | 171.5 |
| C-2′ | 112.9 | 114.1 | | 171.7 |
| C-3′ | 148.6 | 152.5 | | 171.7 |
| C-4′ | 147.2 | 141.1 | | 172.1 |
| C-5′ | 115.5 | 120.5 | | 172.7 |
| C-6′ | 121.7 | 123.5 | | 173.0 |
| C-1″ | 133.8 | 135.3 | | |
| C-2″ | 116.3 | 117.2 | | |
| C-3″ | 150.3 | 151.8 | | |
| C-4″ | 146.7 | 146.8 | | |
| C-5″ | 117.7 | 121.8 | | |
| C-6″ | 124.2 | 124.5 | | |
| 3′-*O*-Me | 56.7 | 57.2 | | |
| 3″-*O*-Me | 56.4 | 56.8 | | |
| Glc-1‴ | 102.9 | 102.1 | | |
| Glc-2‴ | 74.8 | 73.3 | | |
| Glc-3‴ | 77.8 | 74.6 | | |
| Glc-4‴ | 71.3 | 70.3 | | |
| Glc-5‴ | 78.2 | 73.5 | | |
| Glc-6‴ | 62.5 | 62.0 | | |

also proceeded stereoselectively to provide thermodynamically favored addition products **1**, **2** and **3** having the less hindered 11α-configurated L-proline or L-asparagine side chain. Furthermore, we examined the Michael type addition reaction with other amino acids. Treatment of **7** or **8** with L-arginine in $Et_3N$ and 70% aqueous ethanol selectively gave **16** (61% from **7**) or **18** (59% from **8**), which is attached to not the guanidine part but the α-amino acid group (Charts 3 and 4). Treatment of **8** with glycine, L-leucine, L-phenylalanine, L-serine, or L-methionine was also found to give each 11α-derivative linkaged at the α-amino group. However, treatment of **7** and **8** with L-cysteine furnished **17** and **19**, which were adducted between the thiol group and the α-methylene-γ-lactone part. The structures of those synthetic conjugates (**16**–**24**) were determined on the basis of chemical and physicochemical evidence, which included the NOE observation between the 11-proton and the 6-proton (**16**–**24**) and acid hydrolysis of the conjugates to the corresponding amino acids and **7** or **8**.

Next, we carried out the chemical synthesis of **4** and **5** from costunolide (**7**) via santamarine (**9**) and reynosin (**10**). That is, epoxidation of **7** with *m*-chloroperbenzoic acid (*m*-CPBA) in dry-$CHCl_3$ furnished costunolide-1,10-epoxide in a 73% yield, which was subsequently treated with $BF_3{\cdot}Et_2O$ in dry-benzene[19] to give **9** and **10** in a ca. 2:1

**Table 4.** Effects of the MeOH ext., saussureamines A–E (**1**–**5**), costunolide (**7**), dehydrocostus lactone (**8**), cetraxate, cimetidine and omeprazole on gastric lesions induced by HCl/EtOH in rats (significantly different from the control group, $*p<0.05$, $**p<0.01$)

| Treatment | Dose (mg/kg, *p.o.*) | *N* | Gastric lesions | |
|---|---|---|---|---|
| | | | Length (mm) | Inhibition (%) |
| Control | – | 6 | 106.0±13.0 | – |
| MeOH ext. | 12.5 | 6 | 25.8±8.8** | 75.7 |
| | 25 | 6 | 11.1±4.2** | 89.5 |
| | 50 | 6 | 1.2±4.5** | 98.9 |
| Cetraxate hydrochloride | 300 | 6 | 8.7±4.5** | 91.8 |
| Control | – | 8 | 72.4±9.0 | – |
| Saussureamine A (**1**) | 25 | 8 | 63.4±10.5 | 12.4 |
| | 50 | 5 | 42.3±8.3* | 41.6 |
| | 100 | 6 | 29.1±16.6* | 59.8 |
| Saussureamine B (**2**) | 25 | 8 | 74.5±15.0 | −2.9 |
| | 50 | 6 | 45.5±7.0* | 37.2 |
| | 100 | 4 | 9.4±3.9** | 87.0 |
| Saussureamine C (**3**) | 50 | 6 | 57.3±16.3* | 20.9 |
| | 100 | 6 | 23.1±5.5** | 68.1 |
| Saussureamine D (**4**) | 100 | 5 | 63.8±13.9 | 11.9 |
| Saussureamine E (**5**) | 100 | 5 | 63.7±16.9 | 12.0 |
| Cetraxate hydrochloride | 300 | 6 | 9.3±8.6** | 87.2 |
| Control | – | 8 | 91.1±13.0 | – |
| Costunolide (**7**) | 2.5 | 7 | 75.7±11.8 | 16.9 |
| | 5 | 6 | 55.0±10.7* | 39.6 |
| | 10 | 6 | 6.2±1.8** | 93.2 |
| Dehydrocostus lactone (**8**) | 2.5 | 8 | 73.6±8.6 | 19.2 |
| | 5 | 7 | 22.3±6.9** | 75.5 |
| | 10 | 6 | 3.5±1.5** | 96.2 |
| Cetraxate hydrochloride | 75 | 6 | 65.5±7.1 | 28.1 |
| | 150 | 5 | 33.8±6.0** | 62.9 |
| | 300 | 5 | 1.6±1.6** | 98.2 |
| Control | – | 7 | 89.1±5.2 | – |
| Cimetidine | 30 | 6 | 63.9±9.1 | 28.3 |
| | 100 | 6 | 51.9±14.0 | 41.8 |
| | 300 | 6 | 20.7±4.4** | 76.8 |
| Omeprazole | 10 | 6 | 68.0±16.2 | 23.7 |
| | 30 | 6 | 21.8±12.8** | 75.5 |
| | 100 | 6 | 0.7±0.5** | 99.2 |

**Table 5.** Effects of saussureamines A–E (**1**–**5**), costunolide (**7**), dehydrocostus lactone (**8**) and cimetidine on gastric lesions induced by water-immersion stress in mice (significantly different from the control group, *$p<0.05$, **$p<0.01$)

| Treatment | Dose (mg/kg, *p.o.*) | *N* | Gastric lesions | |
|---|---|---|---|---|
| | | | Score | Inhibition (%) |
| Control | – | 7 | 3.00±0.00 | – |
| Saussureamine A (**1**) | 100 | 7 | 1.71±0.18** | 43.0 |
| | 200 | 7 | 1.29±0.42** | 57.0 |
| Saussureamine B (**2**) | 200 | 7 | 2.43±0.20 | 19.0 |
| Saussureamine C (**3**) | 200 | 7 | 2.86±0.14 | 4.7 |
| Saussureamine D (**4**) | 200 | 7 | 2.29±0.36 | 23.7 |
| Saussureamine E (**5**) | 200 | 7 | 2.57±0.20 | 14.3 |
| Costunolide (**7**) | 200 | 7 | 2.57±0.30 | 14.3 |
| Dehydrocostus lactone (**8**) | 200 | 7 | 2.43±0.20 | 19.0 |
| Cimetidine | 150 | 7 | 0.71±0.29** | 76.3 |

ratio. Treatment of **9** or **10** with L-proline in EtOH in the presence of $Et_3N$ furnished **4** in a 91% yield, or **5** in an 82% yield (Chart 5).

### Structure of (−)-massoniresinol 4″-*O*-β-D-glucopyranoside (6)

(−)-Massoniresinol 4″-*O*-β-D-glucopyranoside (**6**) was isolated as a white powder with negative optical rotation ($[\alpha]_D^{20}=-67.0°$). The positive-ion FAB-MS of **6** showed a quasimolecular ion peak at *m/z* 577 $(M+Na)^+$, and the high-resolution MS analysis revealed the molecular formula of **6** to be $C_{26}H_{34}O_{13}$. The UV and IR spectra showed absorption bands ascribable to hydroxyl (IR: 3200, 1024 $cm^{-1}$) and aromatic ring [UV: 228 (log ε 3.93), 279 nm (log ε 3.61); IR: 1600, 1510 $cm^{-1}$]. The $^1H$ NMR ($CD_3OD$) and $^{13}C$ NMR (Table 3) spectra of **6** showed signals assignable to a methylene [δ 2.92, 2.97 (ABq, *J*=12 Hz, 4a-$H_2$)], two methoxyl [δ 3.82, 3.83 (both s, 3′ and 3″-OMe)], a methine bearing a oxygen function [δ 5.00 (s, 2-H)], and aromatic rings [δ 6.75 (d, *J*=8 Hz, 5′-H), 6.79 (dd, *J*=2, 8 Hz, 6′-H), 6.83 (dd, *J*=2, 8 Hz, 6″-H), 7.02 (d, *J*=2 Hz, 2′- and 2″-H), 7.09 (d, *J*=8 Hz, 5″-H)] together with β-glucopyranosyl moiety [δ 4.88 (d, *J*=8 Hz, 1‴-H)]. Ordinary acetylation of **6** furnished the hexaacetate **6a**, of which the $^1H$ NMR ($CD_3OD$) and $^{13}C$ NMR (Table 3) spectra showed signals assignable to six acetyl group [δ 1.99, 2.01, 2.01, 2.04, 2.05, 2.06 (each s)]. In addition, methanolysis of **6** with 9% hydrogen chloride in dry-methanol liberated a known lignan, (−)-massoniresinol.[20] Acid hydrolysis of **6** liberated D-glucose, which was identified by GLC analysis of the thiazolidinie derivatives.[21] Furthermore, the $^1H$ and $^{13}C$ NMR spectra of **6** could be analyzed completely by $^{13}C-^1H$ correlation spectroscopy (C–H COSY), and correlation via long-range coupling (COLOC). The heteronuclear multiple bond correlation (HMBC) on **6** showed a long range correlation between the anomeric proton of the D-glucopyranosyl moiety and the 4″-carbon, while the NOE correlation was observed between the anomeric proton and the 5″-proton. This spectral evidence revealed that the D-glucopyranosyl moiety in **6** was attached to the 4″-hydroxyl group of (−)-massoniresinol. Thus, the structure of (−)-massoniresinol 4″-*O*-β-D-glucopyranoside (**6**) was determined as shown.

### Effects of saussureamines A–E (1–5), costunolide (7) and dehydrocostus lactone (8) on acidified ethanol-induced gastric lesions in rats, and on water-immersion stress-induced gastric lesions in mice

Acidified ethanol-induced gastric mucosal lesions is often used as the experimental model for the analysis of cellular protective action.[22–24] Stress-induced gastric ulceration in rats or mice is also used for evaluation of anti-ulcer drugs.[23–26] The results in the present experiment indicated that oral administration of the methanolic extract showed potent inhibitory effect on gastric mucosal lesions induced by 60% ethanol containing 150 mM hydrochloride (HCl/EtOH) in rats, and its potency was stronger than those of reference drugs, cetraxate, cimetidine and omeprazole (Table 4). Three amino acid–sesquiterpene conjugates, saussureamines A (**1**), B (**2**) and C (**3**), showed significant protective effects at doses of 50 and/or 100 mg/kg. Compounds **1** and **2** especially showed more potent effect than a reference drug, cetraxate. Two principal sesquiterpenes of the nonpolar fraction, costunolide (**7**) and dehydrocostus lactone (**8**), also showed much more potent inhibition at doses of 5 and 10 mg/kg than three reference drugs. By comparing the effects of **1** and **2** with those of **7** and **8**, the 13-amino acid moiety was found to reduce the potency of **1** and **2**. Taking into account their potency and contents, these results indicate that **7** and **8** are the principal active constituents of the non-polar fraction and **1**–**3** are some of active principles of the polar fraction for HCl/EtOH-induced gastric lesions.

Saussureamines (**1**–**5**) exhibited weaker gastroprotective effect on the lesions induced by acidified ethanol than nonpolar sesquiterpenes (**7**, **8**). However, saussureamines showed higher-water solubility than **7** and **8**. This property may be important for the traditional use as a stomachic, which was usually made a medical decoction with water.

Effects of **1**–**8** on water-immersion stress-induced gastric mucosal lesions are shown in Table 5. Oral administration of saussureamine A (**1**) showed significant protective effects at doses of 100 and 200 mg/kg. But, **2**–**8** lacked the significant effect on this lesion at a dose of 200 mg/kg, though **2**, **3**, **7** and **8** showed potent inhibitory effect on HCl/EtOH-induced gastric mucosal lesions.

Our results in this study suggested that two sesquiterpenes

(**7**, **8**) and three amino acid–sesquiterpene conjugates (**1**–**3**) contribute to the clinical use of Saussureae Radix to stomachic.

## Bioassay

### HCl/EtOH-induced gastric lesions

Male Wistar rats weighing about 250 g were fasted for 24 h before the experiment. The gastric lesions were induced by oral administration of 150 mM HCl in 60% (v/v) ethanol (HCl/EtOH). One hour later, the animals were sacrificed by ether anesthesia, and then stomach of each rat was removed. The stomach was inflated by injecting 10 ml of 2% formalin and was dipped into 2% formalin to fix the inner and outer layers of the gastric walls. Subsequently, the stomach was incised along the greater curvature and examined for erosions in the glandular portion. The length of each lesion was measured, and the lesion index was expressed as the sum of the length (mm) of these lesions. Each test compound or the corresponding vehicle alone as a control was given orally 1 h before the HCl/EtOH treatment. The volume of each test compound, vehicle, or the ulcerogenic agents was 5 ml/kg body weight. Each test compound was suspended in 2% (w/v) arabic gum solution. Cetraxate hydrochloride, cimetidine and omeprazole were used as reference compounds.

### Water-immersion stress-induced gastric lesions

The method used was the same as previously reported.[23] Briefly, male ddY mice weighing about 25 g were fasted for 24 h. Each animal was put into a vinyl chloride pipe (10 cm long, 2 cm i.d.), covered at the top and bottom with metal mesh, and mice were immersed in water (22°C) vertically to the neck. After 7 h, the animals were sacrificed, and a macroscopic rating of lesions was made according to a scoring system based on the number of lesions and severity in the fundus region (0: no lesion; 1: hemorrhage was observed in stomach, but no visible lesion; 2: from 1 to 5 small lesions; 3: many lesions more than 5). Each compound solution or vehicle alone was given orally 10 min before stressing. The volume of each test compound solution was 10 ml/kg body weight. Cimetidine was used as a reference compound.

### Statistical analysis

Values were expressed as mean±S.E.M. Statistical significance was assessed by one-way analysis of variance following Dunnett's test for parametric data, and Kruskal–Wallis statistics on ranks following Steel's test for non-parametric data.

## Experimental

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter ($l$=5 cm); UV spectra, Shimadzu UV-1200 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrometer; $^1$H NMR spectra, JNM-LA500 (500 MHz), Varian XL-300 (300 MHz) spectrometer; $^{13}$C NMR spectra, JNM-LA500 (125 MHz), Varian XL-300 (75 MHz) spectrometers with tetramethylsilane as an internal standard; MS and high-resolution MS, JEOL JMS-GCMATE mass spectrometer, JEOL JMS-SX 102A mass spectrometer; recycling HPLC SD-O8 chromatography (Japan Analytical Industry Co., Ltd.).

The following experimental conditions were used for chromatography: ordinary phase column chromatography; Silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150–350 mesh), reversed-phase column chromatography; Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100–200 mesh); Amberlite XAD-2 (ORGANO); Sephadex LH-20 (Pharmacia): TLC, pre-coated TLC plates with Silica gel 60F254 (Merck, 0.25 mm) (normal-phase) and Silica gel RP-18 $60F_{254}$ (Merck, 0.25 mm) (reversed-phase); HPTLC, pre-coated TLC plates with Silica gel RP-18 $60WF_{254s}$ (Merck, 0.25 mm) (reversed-phase). Detection was done by spraying with 1% $Ce(SO_4)_2$-10% aqueous $H_2SO_4$, and Ninhydrin reagent followed by heating, and spraying with Dragendorff reagent.

### Extraction and isolation of saussureamines A–E (1–5), (−)-massoniresinol 4″-*O*-β-D-glucopyranoside (6) and known compounds from the dried roots of *Saussurea lappa*

The dried roots of *Saussurea lappa* Clark (40 kg, cultivated in China) was crushed and extracted three times with MeOH under reflux. Evaporation of the solvent under reduced pressure provided the MeOH extract (6.52 kg, 16.3%), and a part of it (2.13 kg) was partitioned in an AcOEt–$H_2O$ (1:1) mixture, and the aqueous layer was further extracted with 1-BuOH. Removal of the solvent under reduced pressure from AcOEt soluble portion (614 g, 4.7%), 1-BuOH soluble portion (171 g, 1.3%), and $H_2O$ soluble portion (10.3%).

The AcOEt soluble portion (100 g) was subjected to ordinary phase silica gel chromatography (3.0 kg, *n*-hexane–AcOEt) to give three fractions [Fr. 1 (10.5 g), Fr. 2 (72.6 g), Fr. 3 (16.9 g)]. Fraction 2 (20.0 g) was purified by ordinary phase silica gel column chromatography (900 g, *n*-hexane–benzene–$CHCl_3$–AcOEt=15:5:5:1→*n*-hexane–benzene–$CHCl_3$=2:1:1) to give costunolide (**7**, 6.3 g, 1.07%), dehydrocostus lactone (**8**, 8.4 g, 1.43%), and the other fraction. The other fraction (5.3 g) was purified by recycling HPLC SO-8 [JAIGEL 1H-2H, 20×600 mm$^2$ i.d., solvent $CHCl_3$, flow rate 3 ml/min, detection RI] to furnish α- and β-costols mixture (29 mg, 0.005%), and (−)-elema-1,3,11(13)-trien-12-ol (12 mg, 0.002%).

The 1-BuOH soluble portion (161 g) was also subjected to Amberlite XAD-2 (3.0 kg, MeOH→$H_2O$) to give four fractions [Fr. 1 (111.7 g), Fr. 2 (2.3 g), Fr. 3 (33.0 g), Fr. 4 (14.0 g)]. Fraction 3 (33.0 g) was purified by ordinary phase silica gel chromatography [1.0 kg, $CHCl_3$–MeOH–$H_2O$=30:3:1 (lower layer)→10:3:1 (lower layer)→65:35:10 (lower layer)→MeOH] to afford eight fractions [Fr. 3-1 (4.12 g), Fr. 3-2 (0.56 g), Fr. 3-3 (0.76 g), Fr. 3-4 (1.12 g), Fr. 3-5 (11.18 g), Fr. 3-6 (0.20 g), Fr. 3-7 (13.68 g), Fr. 3-8 (1.38 g)]. Fr. 3-1-3-3

was applied to reversed-phase column chromatography (MeOH–$H_2O$) and finally Sephadex LH-20 column chromatography (MeOH) to afford picriside B (731 mg, 0.0059%), (−)-olivil 4″-*O*-β-D-glucopyranoside (49 mg, 0.0004%), and syringin (855 mg, 0.0070%). Fr. 3-4 (1.12 g) was also purified on to reversed-phase column chromatography [30 g, (MeOH–$H_2O$)] and finally Sephadex LH-20 column chromatography (MeOH) to give saussureamine A (**1**, 134 mg, 0.0011%) and saussureamine B (**2**, 257 mg, 0.0021%). Fr. 3-5 (11.18 g) was also purified on to reversed-phase column chromatography [300 g, (MeOH–$H_2O$)] and finally Sephadex LH-20 column chromatography (MeOH) to give saussureamine C (**3**, 73 mg, 0.0006%), (−)-massoniresinol 4″-*O*-β-D-glucopyranoside (**6**, 110 mg, 0.0009%). Fr. 3-6 (0.20 g) was also purified by reversed-phase column chromatography [5 g, (MeOH–$H_2O$)] and finally Sephadex LH-20 column chromatography (MeOH) to give saussureamine D (**4**, 12 mg, 0.0001%) and saussureamine E (**5**, 24 mg, 0.0002%).

Above known constituents were identified by comparison of their physical data ($[\alpha]_D$, IR, $^1$H NMR, $^{13}$C NMR) with reported values.[1,9–14]

**Saussureamine A (1):** Dragendorff test positive, colorless prisms from MeOH–$H_2O$, mp 115–117°C, $[\alpha]_D^{20}$=+36.7° (*c*=0.4, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{20}H_{30}NO_4$ $(M+H)^+$: 348.2175. Found: 348.2169. IR (KBr): 3400, 1780, 1630 cm$^{-1}$. $^1$H NMR (300 MHz, pyridine-$d_5$) δ: 1.34, 1.60 (3H each, both s, 14 and 15-$H_3$), 2.56 (1H, m, 11-H), 3.22 (1H, dd, *J*=3, 13 Hz, 13-H), 3.41 (1H, dd, *J*=6, 13 Hz, 13-H), 4.66 (1H, dd, *J*=9, 10 Hz, 6-H), 4.70 (1H, m, 1-H), 4.80 (1H, d, *J*=10 Hz, 5-H). $^{13}$C NMR (75 MHz, pyridine-$d_5$) δc: given in Table 1. Positive-ion FAB-MS: *m/z* 348 $(M+H)^+$.

**Saussureamine B (2):** Dragendorff test positive, a white powder, $[\alpha]_D$=−25.9° (*c*=0.8, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{20}H_{28}NO_4$ $(M+H)^+$: 346.2018. Found: 346.2059. IR (KBr): 3400, 1770, 1640 cm$^{-1}$. $^1$H NMR (300 MHz, pyridine-$d_5$) δ: 2.85 (1H, m, 11-H), 3.14 (1H, dd, *J*=4, 13 Hz, 13-H), 3.38 (1H, dd, *J*=5, 13 Hz, 13-H), 4.00 (1H, dd, *J*=9, 9 Hz, 6-H), 4.70, 4.80 (1H each, both br s, 14-$H_2$), 5.07, 5.33 (1H each, both br s, 15-$H_2$). $^{13}$C NMR (75 MHz, pyridine-$d_5$) δc: given in Table 1. Positive-ion FAB-MS: *m/z* 346 $(M+H)^+$.

**Saussureamine C (3):** Dragendorff and Ninhydrin test positive, white powder, $[\alpha]_D^{20}$=−17.2° (*c*=0.5, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{19}H_{27}N_2O_5$ $(M+H)^+$: 363.1920. Found: 363.1917. IR (KBr): 3200, 1760, 1675, 1640 cm$^{-1}$. $^1$H NMR (300 MHz, pyridine-$d_5$) δ: 2.67 (1H, m, 11-H), 3.23 (m, 13-H), 3.45 (1H, dd, *J*=6, 13 Hz, 13-H), 3.96 (1H, dd, *J*=9, 9 Hz, 6-H), 4.70, 4.82 (1H each, both br s, 14-$H_2$), 5.06, 5.31 (1H each, both br s, 15-$H_2$). $^{13}$C NMR (75 MHz, pyridine-$d_5$) δc: given in Table 1. Positive-ion FAB-MS: *m/z* 363 $(M+H)^+$, 385 $(M+Na)^+$. Negative-ion FAB-MS: *m/z* 361 $(M-H)^-$.

**Saussureamine D (4):** Dragendorff test positive, colorless needles from MeOH–$H_2O$, mp 235–237°C, $[\alpha]_D^{20}$=+13.3°(*c*=0.3, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{20}H_{30}NO_5$ $(M+H)^+$: 364.2124. Found: 364.2148. IR (KBr): 3200, 1765, 1634 cm$^{-1}$. $^1$H NMR (300 MHz, pyridine-$d_5$) δ: 1.07, 1.94 (3H each, both s, 14 and 15-$H_3$), 2.10 (1H, m, 5-H), 2.35 (1H, m, 7-H), 2.70 (1H, m, 11-H), 3.12 (1H, dd, *J*=4, 13 Hz, 13-H), 3.48 (1H, dd, *J*=6, 13 Hz, 13-H), 3.81 (1H, dd, *J*=6, 10 Hz, 1-H), 4.07 (1H, dd, *J*=10, 10 Hz, 6-H), 5.36 (1H, br s, 3-H). $^{13}$C NMR (75 MHz, pyridine-$d_5$) δc: given in Table 1. Positive-ion FAB-MS: *m/z* 370 $(M+Li)^+$. Negative-ion FAB-MS: *m/z* 362 $(M-H)^-$.

**Saussureamine E (5):** Dragendorff test positive, colorless needles from MeOH–$H_2O$, mp 150–153°C, $[\alpha]_D$=+33.7° (*c*=0.9, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{20}H_{30}NO_5$ $(M+H)^+$: 364.2124. Found: 364.2117. IR (KBr): 3200, 1755, 1630 cm$^{-1}$. $^1$H NMR (300 MHz, pyridine-$d_5$) δ: 1.03 (3H, s, 14-$H_3$), 2.10 (1H, m, 5-H), 2.13 (1H, m, 7-H), 2.78 (1H, ddd, *J*=5, 5, 12 Hz, 11-H), 3.15 (1H, dd, *J*=5, 13 Hz, 13-H), 3.48 (1H, dd, *J*=5, 13 Hz, 13-H), 3.65 (1H, m, 1-H), 4.22 (1H, dd, *J*=10, 10 Hz, 6-H), 4.99, 5.03 (1H each, both br s, 15-$H_2$). $^{13}$C NMR (75 MHz, pyridine-$d_5$) δc: given in Table 1. Positive-ion FAB-MS: *m/z* 364 $(M+H)^+$.

**(−)-Massoniresinol 4″-*O*-β-D-glucopyranoside (6):** A white powder, $[\alpha]_D^{20}$=−67.0° (*c*=1.0, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{26}H_{34}O_{13}Na$ $(M+Na)^+$: 577.1898. Found: 577.1917. UV (MeOH, nm, log ε): 228 (3.93), 279 (3.61). IR (KBr): 3200, 1600, 1510, 1024 cm$^{-1}$. $^1$H NMR (300 MHz, $CD_3OD$) δ: 2.92, 2.97 (2H, ABq, *J*=12 Hz, 4a-$H_2$), 3.82, 3.83 (3H each, both s, 3′ and 3″-OMe), 4.88 (1H, d, *J*=8 Hz, 1‴-H), 5.00 (1H, s, 2-H), 6.75 (1H, d, *J*=8 Hz, 5′-H), 6.79 (1H, dd, *J*=2, 8 Hz, 6′-H), 6.83 (1H, dd, *J*=2, 8 Hz, 6″-H), 7.02 (2H, d, *J*=2 Hz, 2′ and 2″-H), 7.09 (1H, d, *J*=8 Hz, 5″-H). $^{13}$C NMR (75 MHz, $CD_3OD$) δc: given in Table 5. Positive-ion FAB-MS: *m/z* 577 $(M+Na)^+$.

**Acetylation of saussureamine D (4)**

A solution of **4** (5 mg, 0.014 mmol) in pyridine (1.0 ml) was treated with $Ac_2O$ (0.8 ml) and the mixture was stirred at room temperature for 8 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was successively washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$, and brine, then dried over $MgSO_4$ powder and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was purified by silica gel column chromatography [200 mg, $CHCl_3$–MeOH–$H_2O$=10:3:1 (lower layer)] to give **4a** (6 mg, quant.).

**4a:** Dragendorff test positive, white powder, $[\alpha]_D^{20}$=+12.9° (*c*=0.1, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{22}H_{32}NO_6$ $(M+H)^+$: 406.2229. Found: 406.2222. IR (KBr): 3500, 1760, 1740, 1640 cm$^{-1}$. $^1$H NMR (300 MHz, pyridine-$d_5$) δ: 0.94, 1.86 (3H each, both s, 14 and 15-$H_3$), 2.04 (3H, s,–$COCH_3$), 3.17 (1H, dd, *J*=5, 13 Hz, 13-H), 3.30 (1H, ddd, *J*=5, 5, 12 Hz, 11-H), 3.44 (1H, dd, *J*=5, 13 Hz, 13-H), 3.99 (1H, dd, *J*=10, 10 Hz, 6-H), 4.97 (1H, dd, *J*=7, 10 Hz, 1-H), 5.21 (1H, br s, 3-H). $^{13}$C NMR (75 MHz, pyridine-$d_5$) δc: given in Table 1. Positive-ion FAB-MS: *m/z* 428 $(M+Na)^+$.

### Diethylamine addition reaction of dehydrocostus lactone (8)

A solution of **8** (30 mg, 0.13 mmol) in EtOH (2.0 ml) were treated with diethylamine (200 μl) and the mixture was heated under reflux for 8 h. After cooling, the reaction mixture was evaporated under reduced pressure and then furnished a residue, which was purified by silica gel column chromatography [1.5 g, *n*-hexane–AcOEt=3:1] to give **11** (27 mg, 69%).

**11:** Colorless oil, $[\alpha]_D^{27}=+41.0°$ ($c$=1.1, $CHCl_3$). High-resolution FAB-MS: Calcd for $C_{19}H_{30}NO_2$ $(M+H)^+$: 304.2277. Found: 304.2284. IR (film): 1771, 1642 $cm^{-1}$. $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$: 1.01 [6H, dd, $J$=7.0, 7.1 Hz, $-N(CH_2CH_3)_2$], 1.29 (1H, dddd, $J$=4.9, 5.2, 11.3, 17.7 Hz, 8-H), [1.86 (1H, dddd, $J$=5.8, 8.8, 13.4, 18.8 Hz), 1.95 (1H, dddd, $J$=2.8, 7.9, 9.1, 18.8 Hz), 2-$H_2$], 2.04 (1H, ddd, $J$=5.2, 12.2, 17.4 Hz, 9-H), 2.24 (1H, dddd, $J$=3.4, 4.9, 11.3, 14.3 Hz, 7-H), 2.34 (1H, m, 11-H), 2.37 (1H, m, 8-H), 2.43 (1H, m, 9-H), 2.51 (2H, m, 3-$H_2$), [2.46 (2H, dd, $J$=7.1, 13.3 Hz), 2.58 (2H, dd, $J$=7.0, 13.3 Hz), $-N(CH_2CH_3)_2$], [2.66 (1H, dd, $J$=7.3, 13.7 Hz), 2.85 (1H, dd, $J$=4.9, 13.7 Hz), 13-$H_2$], 2.80 (1H, dd, $J$=8.6, 9.4 Hz, 5-H), 2.90 (1H, m, 1-H), 3.92 (1H, dd, $J$=9.4, 9.4 Hz, 6-H), 4.76, 4.86 (1H each, both s, 14-$H_2$), 5.04, 5.20 (1H each, both d, $J$=2.1 Hz, 15-$H_2$). $^{13}C$ NMR (125 MHz, $CHCl_3$) $\delta$c: given in Table 2. Positive-ion FAB-MS: $m/z$ 304 $(M+H)^+$.

### 2-Mercaptoethanol addition reaction of dehydrocostus lactone (8)

A solution of **8** (30 mg, 0.13 mmol) in EtOH (2.0 ml) were treated with 2-mercaptoethanol (50 mg, 0.65 mmol) in the presence of $Et_3N$ (200 μl) and the mixture was heated under reflux for 1 h. After cooling, the reaction mixture was evaporated under reduced pressure and then furnished a residue, which was purified by silica gel column chromatography [1.5 g, *n*-hexane–AcOEt=3:1→1:1] to give **12** (30 mg, 75%).

**12:** Colorless oil, $[\alpha]_D^{30}=+20.4°$ ($c$=2.9, $CHCl_3$). High-resolution FAB-MS: Calcd for $C_{17}H_{25}O_3S$ $(M+H)^+$: 309.1525. Found: 309.1515. IR (film): 3456, 1799, 1642 $cm^{-1}$. $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$: 1.34 (1H, dddd, $J$=5.2, 7.9, 11.6, 16.8 Hz, 8-H), 1.86, 1.94 (1H each, both m, 2-$H_2$), 2.07 (1H, ddd, $J$=5.2, 12.9, 17.1 Hz, 9-H), 2.22 (1H, dddd, $J$=3.7, 4.0, 12.9, 16.8 Hz, 8-H), 2.38 (1H, br ddd, 7-H), 2.48 (1H, m, 9-H), 2.52 (2H, m, 3-$H_2$), 2.54 (1H, m, 11-H), 2.78 (2H, m, -S$CH_2$-), 2.84 (1H, dd, $J$=7.4, 9.8 Hz, 5-H), 2.91 (1H, ddd, $J$=4.3, 7.4, 12.9 Hz, 1-H), 2.95 (2H, dd like, 13-$H_2$), 3.79 (2H, br s, -$SCH_2CH_2$OH), 3.96 (1H, dd, $J$=9.4, 9.8 Hz, 6-H), 4.79, 4.88 (1H each, both s, 14-$H_2$), 5.05, 5.10 (1H each, both br s, 15-$H_2$). $^{13}C$ NMR (125 MHz, $CHCl_3$) $\delta$c: given in Table 2. Positive-ion FAB-MS: $m/z$ 309 $(M+H)^+$.

### Methanol addition reaction of dehydrocostus lactone (8)

A solution of **8** (150 mg, 0.65 mmol) in 0.1% NaOMe–MeOH (5.0 ml) was stirred at room temperature for 8 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was successively washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$, and brine, then dried over $MgSO_4$ powder and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was purified by silica gel column chromatography [2.0 g, *n*-hexane–AcOEt=7:1] to give **13** (150.2 mg, 88%), and **14** (9 mg, 5%).

**13:** Colorless oil, $[\alpha]_D^{25}=+24.6°$ ($c$=13.2, $CHCl_3$). High-resolution EI-MS: Calcd for $C_{16}H_{22}O_3$ $(M^+)$: 262.1569. Found: 262.1567. IR (film): 1771, 1642 $cm^{-1}$. $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$: 1.32 (1H, dddd, $J$=4.9, 6.4, 11.3, 13.0 Hz, 8-H), [1.86 (1H, dddd, $J$=4.2, 5.5, 8.9, 18.8 Hz), 1.95 (1H, dddd, $J$=3.9, 5.1, 11.7, 18.8 Hz), 2-$H_2$], 2.07 (1H, ddd, $J$=5.2, 12.2, 17.4 Hz, 9-H), 2.19 (1H, dddd, $J$=3.5, 5.0, 8.4, 13.0 Hz, 8-H), 2.38 (1H, m, 11-H), 2.44 (1H, m, 7-H), 2.49 (1H, m, 9-H), 2.51 (2H, m, 3-$H_2$), 2.83 (1H, dd, $J$=8.8, 9.2 Hz, 5-H), 2.90 (1H, ddd, $J$=3.9, 8.8, 8.9 Hz, 1-H), 3.37 (3H, s, $-OCH_3$), [3.63 (1H, dd, $J$=3.3, 9.8 Hz), 3.70 (1H, dd, $J$=4.3, 9.8 Hz), 13-$H_2$], 3.93 (1H, dd, $J$=9.2, 9.5 Hz, 6-H), 4.76, 4.87 (1H each, both s, 14-$H_2$), 5.03, 5.19 (1H each, both d, $J$=1.5 Hz, 15-$H_2$). $^{13}C$ NMR (125 MHz, $CHCl_3$) $\delta$c: given in Table 2. EI-MS: $m/z$ 262 $(M^+, 3)$, 230 $(M^+-CH_4O$, 19), 158 (100).

**14:** Colorless oil, $[\alpha]_D^{26}=+56.4°$ ($c$=0.6, $CHCl_3$). High-resolution FAB-MS: Calcd for $C_{16}H_{22}O_3$ $(M^+)$: 262.1569. Found: 262.1573. IR (film): 1773, 1653 $cm^{-1}$. $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$: 1.59 (1H, br ddd, 8-H), [1.87 (1H, dddd, $J$=4.6, 5.2, 12.6, 17.4 Hz), 1.94 (1H, m), 2-$H_2$], 1.96 (1H, m, 8-H), 2.03 (1H, ddd, $J$=5.2, 12.6, 17.4 Hz, 9-H), 2.47 (1H, m, 7-H), 2.50 (1H, m, 9-H), 2.51 (2H, m, 3-$H_2$), 2.67 (1H, ddd, $J$=2.5, 3.7, 9.5 Hz, 11-H), 2.76 (1H, dd, $J$=8.3, 9.8 Hz, 5-H), 2.89 (1H, ddd, $J$=4.6, 8.3, 9.0 Hz, 1-H), 3.29 (3H, s, $-OCH_3$), [3.58 (1H, dd, $J$=2.5, 9.5 Hz), 3.73 (1H, dd, $J$=3.7, 9.5 Hz), 13-$H_2$], 4.13 (1H, dd, $J$=9.8, 9.9 Hz, 6-H), 4.78, 4.87 (1H each, both s, 14-$H_2$), [5.04 (1H, d, $J$=1.2 Hz), 5.22 (1H, d, $J$=1.6 Hz), 15-$H_2$]. $^{13}C$ NMR (125 MHz, $CHCl_3$) $\delta$c: given in Table 2. EI-MS: $m/z$ 262 $(M^+, 6)$, 230 $(M^+-CH_4O$, 32), 158 (100).

### Treatment of costunolide (7) with 2-mercaptoethanol and diethylamine

A solution of **7** (30 mg, 0.13 mmol) in EtOH (2.0 ml) were treated with 2-mercaptoethanol (50 mg, 0.65 mmol) and diethylamine (47 mg, 0.65 mmol) and the mixture was heated under reflux for 1 h. After cooling, the reaction mixture was evaporated under reduced pressure furnished a residue, which was purified by silica gel column chromatography [1.5 g, *n*-hexane–AcOEt=3:1→1:1] to give **15** (29 mg, 71%).

**15:** Colorless oil, $[\alpha]_D^{30}=+84.8°$ ($c$=2.7, $CHCl_3$). High-resolution positive-ion EI-MS: Calcd for $C_{17}H_{26}O_3S$ $(M)^+$: 310.1603. Found: 310.1615. IR (film): 3475, 1779, 1667 $cm^{-1}$. $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$: 1.42 (3H, s, 14-$H_3$), 1.69 (3H, d, $J$=1.2 Hz, 15-$H_3$), [1.64 (1H, br ddd), 1.92 (1H, dddd, $J$=1.2, 6.1, 7.0, 15.0 Hz), 8-$H_2$], 2.05, 2.31 (1H each, both m, 3-$H_2$), 2.10, 2.37 (1H each, both br dd, 9-$H_2$), 2.15 (1H, m, 7-H), 2.18, 2.28 (1H each, both m, 2-$H_2$), 2.58 (1H, ddd, $J$=4.0, 4.6, 12.6 Hz, 11-H), 2.79 (2H, m, $-SCH_2-$), [2.95 (1H, dd, $J$=4.6, 13.7 Hz), 3.03

(1H, dd, $J$=4.0, 13.7 Hz), 13-$H_2$], 3.79 (2H, m, $-SCH_2CH_2OH$), 4.60 (1H, dd, $J$=9.2, 9.7 Hz, 6-H), 4.69 (1H, d, $J$=9.7 Hz, 5-H), 4.85 (1H, br d, 1-H). $^{13}C$ NMR (125 MHz, pyridine-$d_5$) δc: given in Table 2. EI-MS: $m/z$ 310 ($M^+$, 6), 292 ($M^+-H_2O$, 10), 83 (100).

**Synthesis of saussureamine A (1)**

A solution of **7** (500 mg, 2.2 mmol) in EtOH (6.0 ml) was treated with L-proline (500 mg, 4.4 mmol) in the presence of $Et_3N$ (300 μl) and the mixture was heated under reflux for 1 h. After cooling, the reaction mixture was evaporated under reduced pressure and then furnished a residue, which was purified by silica gel column chromatography [10 g, $CHCl_3$–MeOH–$H_2O$=8:3:1 (lower layer)] to give **1** (568 mg, 76%).

**Syntheses of saussureamines B (2) and C (3)**

A solution of **8** (500 mg, 2.2 mmol) in EtOH (6.0 ml) was treated with L-proline (500 mg, 4.4 mmol) in the presence of $Et_3N$ (300 μl) and the mixture was heated under reflux for 1 h. After cooling, the reaction mixture was evaporated under reduced pressure and then furnished a residue, which was purified by silica gel column chromatography [10 g, $CHCl_3$–MeOH–$H_2O$=8:3:1 (lower layer)] to give **2** (525 mg, 70%). On the other hand, a solution of **8** (500 mg, 2.2 mmol) in 70% aqueous EtOH (20 ml) was treated with L-asparagine (573 mg, 4.4 mmol) in the presence of $Et_3N$ (220 μl) and the mixture was heated under reflux for 2 h. Through a similar procedure, **3** (378 mg, 48%) was prepared from **8** (500 mg, 2.2 mmol).

**Syntheses of the other costunolide (7) or dehydrocostus lactone (8)-amino acid conjugates (16–24)**

A solution of **7** or **8** (150 mg, 0.65 mmol, respectively) in 70% aqueous EtOH (3.0 ml) was treated with L-arginine (350 mg, 2.0 mmol) in the presence of $Et_3N$ (300 μl) and the mixture was heated under reflux for 12 h. After cooling, the reaction mixture was evaporated under reduced pressure and then furnished a residue, which was purified by silica gel column chromatography [10 g, $CHCl_3$–MeOH–$H_2O$=7:3:1 (lower layer)] to give **16** [81 mg, 61% (conversion yield)] and recovered **7** (74 mg), or **18** [82 mg, 59% (conversion yield)] and recovered **8** (71 mg). On the similar procedure, a solution of **7** or **8** (150 mg, 0.65 mmol, respectively) in 70% aqueous EtOH (3.0 ml) was treated with L-cysteine (240 mg, 2.0 mmol) in the presence of $Et_3N$ (300 μl) and the mixture was heated under reflux for 1 h, then purified by silica gel column chromatography [10 g, $CHCl_3$–MeOH–$H_2O$=65:35:10 (lower layer)] to furnish **17** [130 mg, 82% (conversion yield)] and recovered **7** (45 mg), or **19** (164 mg, 72%).

In addition, a solution of **8** (150 mg, 0.65 mmol) in EtOH (3.0 ml) was treated with following amino acids [glycine (150 mg, 2.0 mmol), L-leucine (262 mg, 2.0 mmol), L-phenylalanine (330 mg, 2.0 mmol), L-serine (210 mg, 2.0 mmol), and L-methionine (298 mg, 2.0 mmol)] in the presence of $Et_3N$ (300 μl) and the mixture was heated under reflux for 2–10 h. After cooling, the reaction mixture was evaporated under reduced pressure furnished a residue, which was purified by silica gel column chromatography [10 g, $CHCl_3$–MeOH–$H_2O$=7:3:1 (lower layer) or 65:35:10 (lower layer)] furnish **20** (165 mg, 83%), **21** (183 mg, 89%), **22** (193 mg, 75%), **23** (166 mg, 76%), and **24** [102 mg, 54% (conversion yield), recovered **8** (35 mg)], respectively.

**16:** A white powder, $[\alpha]_D^{26}$=+45.3° ($c$=0.5, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{21}H_{35}N_4O_4$ $(M+H)^+$: 407.2659. Found: 407.2668. IR (KBr): 3430, 1768, 1655, 1638 $cm^{-1}$. $^1H$ NMR (500 MHz, pyridine-$d_5$) δ: 1.34, 1.58 (3H each, both s, 14 and 15-$H_3$), 1.59 (1H, m, 8-H), 1.90 (1H, m, 4′-H), 1.92 (1H, m, 3-H), 1.97 (1H, m, 8-H), 1.98 (2H, m, 3′-$H_2$), 2.05 (2H, m, 2 and 4′-H), 2.08 (1H, m, 7-H), 2.10 (1H, m, 9-H), 2.12 (1H, m, 3-H), 2.17 (1H, m, 2-H), 2.28 (1H, ddd, $J$=5.4, 12.8, 13.5 Hz, 9-H), 2.65 (1H, ddd, $J$=3.4, 6.5, 11.0 Hz, 11-H), [3.08 (1H, dd, $J$=3.4, 12.0 Hz), 3.31 (1H, dd, $J$=6.5, 12.0 Hz), 13-$H_2$], [3.33 (1H, m), 3.63 (1H, br d), 5′-$H_2$], 3.57 (1H, br s, 2′-H), 4.64 (1H, dd, $J$=9.2, 9.5 Hz, 6-H), 4.70 (1H, d, $J$=9.5 Hz, 5-H), 4.78 (1H, dd, $J$=3.9, 11.6 Hz, 1-H). $^{13}C$ NMR (125 MHz, pyridine-$d_5$) δc: given in Table 1. Positive-ion FAB-MS: $m/z$ 407 $(M+H)^+$. Negative-ion FAB-MS: $m/z$ 405 $(M-H)^-$.

**17:** A white powder, $[\alpha]_D^{27}$=+44.9° ($c$=0.8, DMSO). High-resolution positive-ion FAB-MS: Calcd for $C_{18}H_{28}NO_4S$ $(M+H)^+$: 354.1739. Found: 354.1727. IR (KBr): 3456, 1766, 1655, 1638 $cm^{-1}$. $^1H$ NMR (500 MHz, DMSO-$d_6$) δ: 1.38, 1.64 (3H each, both s, 14 and 15-$H_3$), 1.68, 1.90 (1H each, both m, 8-$H_2$), 1.96, 2.23 (1H each, both m, 3-$H_2$), 1.98 (1H, m, 7-H), 2.00, 2.28 (1H each, both m, 9-$H_2$), 2.09, 2.25 (1H each, both m, 2-$H_2$), 2.73 (1H, ddd, $J$=4.3, 5.2, 12.2 Hz, 11-H), 2.81 (1H, dd, $J$=7.9, 14.0 Hz, 3′-H), [2.87 (1H, dd, $J$=4.3, 14.1 Hz), 2.94 (1H, dd, $J$=5.2, 14.1 Hz), 13-$H_2$], 3.06 (1H, dd, $J$=3.9, 14.0 Hz, 3′-H), 3.37 (1H, br s, 2′-H), 4.65 (1H, d, $J$=9.8 Hz, 5-H), 4.74 (1H, dd, $J$=9.1, 9.8 Hz, 6-H), 4.80 (1H, dd, $J$=4.9, 14.6 Hz, 1-H). $^{13}C$ NMR (125 MHz, DMSO-$d_5$) δc: given in Table 1. Positive-ion FAB-MS: $m/z$ 354 $(M+H)^+$. Negative-ion FAB-MS: $m/z$ 352 $(M-H)^-$.

**18:** A white powder, $[\alpha]_D^{27}$=−2.4° ($c$=0.9, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{21}H_{33}N_4O_4$ $(M+H)^+$: 405.2502. Found: 405.2493. IR (KBr): 3379, 1763, 1663, 1630 $cm^{-1}$. $^1H$ NMR (500 MHz, pyridine-$d_5$) δ: 1.27 (1H, dddd, $J$=6.1, 9.1, 11.9, 16.1 Hz, 8-H), [1.70 (dddd, $J$=4.9, 5.5, 9.5, 17.7 Hz), 1.80 (dddd, $J$=7.6, 9.4, 10.9, 17.7 Hz), 2-$H_2$], 2.00, 2.02 (2H each, both m, 3′-$H_2$ and 4′-$H_2$), 2.03, 2.33 (1H each, both m, 9-$H_2$), 2.21 (1H, br dd, 8-H), 2.35 (1H, m, 7-H), 2.40 (2H, m, 3-$H_2$), 2.74 (1H, dd, $J$= 7.6, 9.5 Hz, 5-H), 2.75 (1H, m, 11-H), 2.81 (1H, ddd, $J$=4.9, 6.9, 10.9 Hz, 1-H), 3.09 (1H, dd, $J$=4.3, 12.2 Hz, 13-H), 3.33 (1H, m, 5′-H), 3.35 (1H, dd-like, 13-H), 3.47 (1H, br d, 5′-H), 3.63 (1H, br s, 2′-H), 3.89 (1H, dd, $J$=9.1, 9.5 Hz, 6-H), 4.68, 4.81 (1H each, both s, 14-$H_2$), 5.03, 5.23 (1H each, both s, 15-$H_2$). $^{13}C$ NMR (125 MHz, pyridine-$d_5$) δc: given in Table 1. Positive-ion FAB-MS: $m/z$ 405 $(M+H)^+$. Negative-ion FAB-MS: $m/z$ 403 $(M-H)^-$.

**19:** A white powder, $[\alpha]_D^{27}$=+15.6° ($c$=0.2, DMSO).

High-resolution positive-ion FAB-MS: Calcd for $C_{18}H_{26}NO_4S$ $(M+H)^+$: 352.1583. Found: 352.1571. IR (KBr): 3420, 1782, 1655 $cm^{-1}$. $^1H$ NMR (500 MHz, DMSO-$d_6$) $\delta$: 1.34 (1H, dddd, $J$=4.7, 5.2, 11.6, 17.1 Hz, 8-H), [1.77 (dddd, $J$=3.7, 5.2, 8.9, 18.6 Hz), 1.90 (dddd, $J$=7.9, 9.4, 13.4, 18.6 Hz), 2-$H_2$], 2.03 (1H, ddd, $J$=5.2, 12.2, 17.2 Hz, 9-H), 2.17 (1H, dddd, $J$=3.7, 4.6, 12.2, 17.1 Hz, 8-H), 2.32 (1H, dddd, $J$=3.7, 9.5, 11.6, 14.6 Hz, 7-H), 2.39 (1H, ddd, $J$=4.6, 4.7, 17.2 Hz, 9-H), 2.47 (2H, m, 3-$H_2$), 2.73 (1H, ddd, $J$=4.9, 8.3, 14.6 Hz, 11-H), 2.76 (1H, dd, $J$=8.3, 9.7 Hz, 5-H), [2.79 (1H, dd, $J$=8.3, 13.8 Hz), 2.93 (1H, dd, $J$=4.9, 13.8 Hz), 13-$H_2$], [2.81 (1H, dd, $J$=5.2, 14.1 Hz), 3.03 (1H, dd, $J$=3.7, 14.1 Hz), 3′-$H_2$], 2.90 (1H, ddd, $J$=5.2, 8.3, 13.4 Hz, 1-H), 3.32 (1H, br dd, 2′-H), 3.94 (1H, dd, $J$=9.5, 9.7 Hz, 6-H), 4.73, 4.85 (1H each, both s, 14-$H_2$), 4.97, 5.03 (1H each, both br s, 15-$H_2$). $^{13}C$ NMR (125 MHz, DMSO-$d_6$) $\delta$c: given in Table 1. Positive-ion FAB-MS: $m/z$ 352 $(M+H)^+$. Negative-ion FAB-MS: $m/z$ 350 $(M-H)^-$.

**20:** A white powder, $[\alpha]_D^{27}=-6.4°$ ($c$=0.2, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{17}H_{24}NO_4$ $(M+H)^+$: 306.1706. Found: 306.1710. IR (KBr): 3368, 1774, 1655 $cm^{-1}$. $^1H$ NMR (500 MHz, pyridine-$d_5$) $\delta$: 1.29 (1H, m, 8-H), 1.76 (2H, m, 2-$H_2$), 1.95 (1H, ddd, $J$=5.5, 12.5, 17.1 Hz, 9-H), 2.15, 2.39 (1H each, both m, 3-$H_2$), 2.35 (1H, m, 9-H), 2.39 (1H, m, 8-H), 2.52 (1H, m, 7-H), 2.55 (1H, m, 11-H), 2.75 (1H, br d, 1-H), 2.75 (1H, br d, 5-H), 3.18 (2H, br s, 13-$H_2$), 3.73 (2H, s, 2′-$H_2$), 3.96 (1H, dd, $J$=8.8, 8.9 Hz, 6-H), 4.73, 4.83 (1H each, both s, 14-$H_2$), 5.06, 5.33 (1H each, both s, 15-$H_2$). $^{13}C$ NMR (125 MHz, pyridine-$d_5$) $\delta$c: given in Table 1. Positive-ion FAB-MS: $m/z$ 306 $(M+H)^+$.

**21:** A white powder, $[\alpha]_D^{26}=+15.2°$ ($c$=0.4, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{21}H_{32}NO_4$ $(M+H)^+$: 362.2331. Found: 362.2340. IR (KBr): 3370, 1778, 1655 $cm^{-1}$. $^1H$ NMR (500 MHz, pyridine-$d_5$) $\delta$: 0.97, 0.97 (3H each, both d, $J$=6.8 Hz, 5′ and 6′-$H_3$), 1.27 (1H, m, 8-H), 1.69, 1.81 (1H each, both m, 3′-$H_2$), 1.77 (2H, m, 2-$H_2$), 1.96 (1H, m, 9-H), 2.07 (1H, m, 4′-H), 2.14 (1H, m, 8-H), 2.36 (1H, m, 9-H), 2.38, 2.45 (1H each, both m, 3-$H_2$), 2.42 (1H, m, 7-H), 2.54 (1H, ddd, $J$=4.9, 5.8, 11.3 Hz, 11-H), 2.78 (1H, m, 1-H), 2.78 (1H, m, 5-H), [3.04 (1H, dd, $J$=4.9, 12.2 Hz), 3.35 (1H, dd, $J$=5.8, 12.2 Hz), 13-$H_2$], 3.67 (1H, dd, $J$=6.1, 8.5 Hz, 2′-H), 4.74, 4.84 (1H each, both s, 14-$H_2$), 5.07, 5.33 (1H each, both br s, 15-$H_2$). $^{13}C$ NMR (125 MHz, pyridine-$d_5$) $\delta$c: given in Table 1. Positive-ion FAB-MS: $m/z$ 362 $(M+H)^+$. Negative-ion FAB-MS: $m/z$ 360 $(M-H)^-$.

**22:** A white powder, $[\alpha]_D^{26}=-4.6°$ ($c$=0.2, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{24}H_{30}NO_4$ $(M+H)^+$: 396.2175. Found: 396.2170. IR (KBr): 3328, 1774, 1655 $cm^{-1}$. $^1H$ NMR (500 MHz, pyridine-$d_5$) $\delta$: 1.19 (1H, dddd, $J$=4.8, 7.0, 12.8, 17.4 Hz, 8-H), 1.76 (2H, m, 2-$H_2$), 1.89 (1H, dd like, 9-H), 2.06 (1H, br dd, 8-H), 2.30 (1H, m, 7-H), 2.34 (1H, m, 9-H), 2.44 (2H, m, 3-$H_2$), 2.47 (1H, m, 11-H), 2.70 (1H, br d, 5-H), 2.72 (1H, br d, 1-H), [3.02 (1H, dd, $J$=5.2, 12.2 Hz), 3.41 (1H, m), 13-$H_2$], [3.13(1H, dd, $J$=7.9, 13.7 Hz), 3.42 (1H, m), 3′-$H_2$], 3.90 (1H, m, 2′-H), 3.91 (1H, dd, $J$=8.8, 9.5 Hz, 6-H), 4.72, 4.83 (1H each, both s, 14-$H_2$), 5.06, 5.31 (1H each, both br s, 15-$H_2$), [7.22 (1H, t, $J$=8.0 Hz), 7.32 (2H, dd, $J$=7.3, 8.0 Hz), 7.46 (2H, d, $J$=7.3 Hz), arom.-H]. $^{13}C$ NMR (125 MHz, pyridine-$d_5$) $\delta$c: given in Table 1. Positive-ion FAB-MS: $m/z$ 396 $(M+H)^+$. Negative-ion FAB-MS: $m/z$ 394 $(M-H)^-$.

**23:** A white powder, $[\alpha]_D^{28}=+13.7°$ ($c$=0.1, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{18}H_{26}NO_5$ $(M+H)^+$: 336.1811. Found: 336.1804. IR (KBr): 3380, 1765, 1655 $cm^{-1}$. $^1H$ NMR (500 MHz, pyridine-$d_5$) $\delta$: 1.24 (1H, m, 8-H), 1.74 (2H, m, 2-$H_2$), 1.93 (1H, ddd, $J$=5.5, 12.2, 12.6 Hz, 9-H), 2.14 (1H, m, 8-H), 2.36 (1H, m, 9-H), 2.40, 2.43 (1H each, both m, 3-$H_2$), 2.44 (1H, m, 7-H), 2.57 (1H, ddd, $J$=4.3, 5.8, 10.9 Hz, 11-H), 2.72 (1H, br d, 1-H), 2.72 (1H, br d, 5-H), [3.18 (1H, dd, $J$=4.3, 12.3 Hz), 3.41 (1H, dd, $J$=5.8, 12.3 Hz), 13-$H_2$], 3.90 (1H, t, $J$=4.9 Hz, 2′-H), 3.95 (1H, dd, $J$=8.2, 9.2 Hz, 6-H), 4.34 (2H, d, $J$=4.9 Hz, 3′-$H_2$), 4.72, 4.82 (1H each, both br s, 14-$H_2$), 5.06, 5.11 (1H each, both br s, 15-$H_2$). $^{13}C$ NMR (125 MHz, pyridine-$d_5$) $\delta$c: given in Table 1. Positive-ion FAB-MS: $m/z$ 336 $(M+H)^+$. Negative-ion FAB-MS: $m/z$ 334 $(M-H)^-$.

**24:** A white powder, $[\alpha]_D^{27}=+20.0°$ ($c$=0.2, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{20}H_{30}NO_4S$ $(M+H)^+$: 380.1895. Found: 380.1890. IR (KBr): 3375, 1778, 1655 $cm^{-1}$. $^1H$ NMR (500 MHz, pyridine-$d_5$) $\delta$: 1.28 (1H, m, 8-H), 1.76 (2H, m, 2-$H_2$), 1.97 (1H, ddd, $J$=5.5, 12.2, 12.8 Hz, 9-H), 2.05 (3H, s, 5′-$H_3$), 2.15 (1H, m, 8-H), 2.15, 2.31 (1H each, both m, 3′-$H_2$), 2.37 (1H, m, 9-H), 2.41 (1H, m, 7-H), 2.43 (2H, m, 3-$H_2$), 2.58 (1H, m, 11-H), 2.78 (1H, br dd, 1-H), 2.78 (1H, br dd, 5-H), 2.86 (2H, dd, $J$=7.0, 7.9 Hz, 4′-$H_2$), [3.05 (1H, dd, $J$=7.3, 12.2 Hz), 3.35 (1H, dd, $J$=6.1, 12.2 Hz), 13-$H_2$], 3.78 (1H, dd, $J$=5.2, 8.0 Hz, 2′-H), 3.96 (1H, dd, $J$=9.1, 9.8 Hz, 6-H), 4.85, 4.94 (1H each, both s, 14-$H_2$), 5.08, 5.18 (1H each, both br s, 15-$H_2$). $^{13}C$ NMR (125 MHz, pyridine-$d_5$) $\delta$c: given in Table 1. Positive-ion FAB-MS: $m/z$ 380 $(M+H)^+$. Negative-ion FAB-MS: $m/z$ 378 $(M-H)^-$.

## Syntheses of saussureamines D (4) and E (5)

A solution of **7** (1160 mg, 5.0 mmol) in $CHCl_3$ (5.0 ml) was treated with *m*-CPBA (880 mg, 5.1 mmol) and the mixture was stirred at 0°C for 30 min. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was successively washed with saturated aqueous $NaHCO_3$ and brine, then dried over $MgSO_4$ powder and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was purified by silica gel column chromatography (50 g, *n*-hexane–AcOEt=2:1) to give costunolide-1,10-epoxide (900 mg, 73%).

A solution of costunolide-1,10-epoxide (800 mg, 3.5 mmol) in benzene (10.0 ml) was treated with $BF_3{\cdot}Et_2O$ (80 μl) and the mixture was stirred at room temperature for 30 min. The reaction mixture was poured into ice-water ant the whole was extracted with AcOEt. The AcOEt extract was successively washed with 5% aqueous $NaHCO_3$ and brine, then dried over $MgSO_4$ powder and filtered. Removal of the solvent from the filtrate under reduced pressure furnished

a residue, which was purified by silica gel column chromatography (40 g, *n*-hexane–AcOEt=2:1) to furnish santamarine (**9**, 520 mg, 65%) and reynosin (10, 278 mg, 35%).

A solution of **9** (300 mg, 1.2 mmol) in EtOH (5.0 ml) was treated with L-proline (280 mg, 2.4 mmol) in the presence of $Et_3N$ (170 μl) and the mixture was heated under reflux for 2 h. After cooling, the reaction mixture was evaporated under reduced pressure furnished a residue, which was purified by reversed-phase column (5 g, MeOH–$H_2O$=30:70) to give **4** (400 mg, 91%). Through a similar procedure, a solution of **10** (270 mg, 1.1 mmol) in EtOH (5.0 ml) was treated with L-proline (260 mg, 2.2 mmol) in the presence of $Et_3N$ (150 μl) and the reaction mixture was heated under reflux for 2 h. Work-up and the residue was purified by reversed-phase column chromatography (5 g, MeOH–$H_2O$=30:70) to give **5** (320 mg, 82%).

### Acid treatment of saussureamines A–E (1–5) and the other amino acid conjugates (16–24)

Saussureamines (**1**–**5**, 4.0 mg each) and the other amino acid conjugates (**16**–**24**, 4.0 mg, each) were treated with 1% aqueous HCl (1.0 ml) at room temperature for 12 h. After cooling, a part of reaction mixture was subject to ordinary phase TLC (1-BuOH–AcOH–$H_2O$=4:2:1) to detect an amino acid (L-proline from **1**, **2**, **4** and **5** and L-asparagine from **3**) by Ninhydrin reagent followed by heating. The reaction mixture was neutralized with Amberlite IRA-400 ($OH^-$ form) and the resin was filtered, and the whole was extracted with AcOEt. The AcOEt extract was dried over $MgSO_4$ powder and filtered to furnish **7** (from **1**, **16**, **17**), **8** (from **2**, **3**, **18**–**24**), **9** (from **4**), and **10** (from **5**), quantitatively. Compounds **7**–**10** were identified by comparison of their physical data ($[\alpha]_D$, $^1H$ NMR) with those of authentic samples.[1,9,16,17]

### Acetylation of (−)-massoniresinol 4″-*O*-β-D-glucopyranoside (6)

A solution of **6** (5 mg, 0.009 mmol) in pyridine (1.0 ml) was treated with $Ac_2O$ (0.8 ml) and the mixture was stirred at room temperature for 12 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract as described above gave a product which was purified by silica gel column chromatography (200 mg, benzene–AcOEt=1:2) to yielded hexaacetate **6a** (7 mg, quant.).

**6a:** A white powder, $[\alpha]_D^{20}=-27.5°$ ($c$=1.0, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{38}H_{46}O_{19}Na$ $(M+Na)^+$: 829.2531. Found: 829.2545. IR (KBr): 1759, 1609, 1512, 1036 $cm^{-1}$. $^1H$ NMR (300 MHz, $CD_3OD$) δ: 1.99, 2.01, 2.01, 2.04, 2.05, 2.06 (3H each, both s, $-COCH_3$), 2.87, 2.94 (2H, ABq, $J$=14 Hz, 4a-$H_2$), 3,80, 3,81 (3H each, both s, 3′ and 3″-OMe), 4.06, 4.11 (2H, ABq, $J$=14 Hz, 3a-$H_2$), 4.84 (1H, d, $J$=8 Hz, 1‴-H), 5.09 (1H, s, 2-H), 6.80 (1H, dd, $J$=2, 8 Hz, 6′-H), 6.92 (1H, dd, $J$=2, 8 Hz, 6″-H), 6.98 (1H, d, $J$=8 Hz, 5′-H), 7.03 (1H, d, $J$=2 Hz, 2′-H), 7.06 (1H, d, $J$=8 Hz, 5′-H), 7.12 (1H, d, $J$=2 Hz, 2″-H). $^{13}C$ NMR (75 MHz, $CD_3OD$) δc: given in Table 3. Positive-ion FAB-MS: $m/z$ 829 $(M+Na)^+$.

### Methanolysis of (−)-massoniresinol 4″-*O*-β-D-glucopyranoside (6)

A solution of (−)-Massoniresinol 4″-*O*-β-D-glucopyranoside (**6**, 5 mg, 0.009 mmol) in 9% dry HCl–MeOH (2.0 ml) was heated under reflux for 3 h. After cooling, the reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was treated with usual manner to give a residue, which was purified by silica gel column chromatography [200 mg, $CHCl_3$–MeOH–$H_2O$=10:3:1 (lower layer)] to furnish (−)-massoniresinol (3 mg, 85%), which were identified by comparison of the IR, $^1H$ NMR, and $^{13}C$ NMR data with reported values.[20]

### Acid hydrolysis (−)-massoniresinol 4″-*O*-β-D-glucopyranoside (6)

A solution of (−)-massoniresinol 4″-*O*-β-D-glucopyranoside (**6**, 2 mg) in 5% aqueous $H_2SO_4$–dioxane (2 ml, 1:1, v/v) was heated under reflux for 3 h. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 ($OH^-$ form) and the resin was filtered. After removal of the solvent in vacuo from the filtrate, the residue was passed through a Sep-Pak C18 cartridge with $H_2O$ and MeOH. The $H_2O$ eluate was concentrated and the residue was treated with L-cysteine methyl ester hydrochloride (4 mg) in pyridine (0.02 ml) at 60°C for 1 h. After reaction, the solution was treated with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA, 0.01 ml) at 60°C for 1 h. The supernatant was then subjected to GLC analysis to identify the derivative of D-glucose. GLC conditions: column, Supeluco STB™-1 (30 m×0.25 mm i.d.); injection temperature, 230°C; detection temperature, 230°C; column temperature, 230°C; He flow rate, 15 ml/min; $t_R$, 24.1 min.[21]

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