Pergamon

Bioorganic & Medicinal Chemistry 8 (2000) 2071–2077

BIOORGANIC & MEDICINAL CHEMISTRY

Alcohol Absorption Inhibitors from Bay Leaf (*Laurus nobilis*): Structure-Requirements of Sesquiterpenes for the Activity

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Received 7 March 2000; accepted 12 May 2000

Abstract—Through a bioassay-guided separation using inhibitory activity on blood ethanol elevation in oral ethanol-loaded rat, various sesquiterpenes having an α-methylene-γ-butyrolactone moiety, costunolide (**1**), dehydrocostus lactone (**2**), zaluzanin D (**3**), reynosin (**4**), santamarine (**5**), 3α-acetoxyeudesma-1,4(15),11(13)-trien-12,6α-olide (**6**) and 3-oxoeudesma-1,4,11(13)-trien-12,6α-olide (**7**), were isolated as the active principle from the leaves of *Laurus nobilis* (bay leaf, laurel). In order to characterize the structure requirement for the activity, several reduction products (**2a**–**2d**) and amino acid adducts (**2e**, **2f**) of the α-methylene-γ-butyrolactone moiety were synthesized from **2** and the inhibitory activities of these sesquiterpenes, together with α-methylene-γ-butyrolactone (**12**) and its related compounds (**13**–**16**), were examined. These results indicated that the γ-butyrolactone or γ-butyrolactol moiety having α-methylene or α-methyl group was essential for the inhibitory activity on ethanol absorption. Since **1**, **2** and **12** showed no significant effect on glucose absorption, these sesquiterpenes appeared to selectively inhibit ethanol absorption. In addition, the acute toxicities of **1** and **2** in a single oral administration were found to be lower than that of **12**.

Introduction

Taking large quantities of alcohol in one time is known to induce an acute alcohol toxicity with acidosis, heart failure and respiratory depression caused by an autonomic nerve and cerebrum dysfunction. Long-term consumption of alcohol in large quantities induces a number of disorders, e.g. hepatopathy, gastrointestinal disorder, chronic pancreatitis, peripheral nerve disorder, myocardosis, hypertension and hematopoiesis disorder. Long-term alcohol consumption not only causes organ dysfunctions but also can cause phrenopathy and have threatening social effects. Violent crimes and traffic accidents caused by alcohol intoxication have increased over the past decade in developed countries. It is also reported that there is a correlation between alcohol consumption, the number of alcoholics and the occurrence of life-threatening assaults.

Benzodiazepines and vitamins are medicated for alcoholic patients in the elimination phase of the cure process to suppress withdrawal.[1] Cyanamide and disulfiram, aldehyde dehydrogenase inhibitors, are also used to decrease the appetite for drinking.[2] However, it is known that benzodiazepines lead to abuse, psychological dependence, and adverse behavioral effects. These drugs can produce physiological dependence when taken chronically, and result in dose escalation.[3] Aldehyde dehydrogenase inhibitors force alcoholics to quit drinking based on the fear of unpleasant reaction elicited after ethanol intake, but these medicines, especially disulfiram, are reported to show serious toxic symptoms such as fulminant hepatitis, optic neuritis, peripheral neuropathy, encephalopathy, or catatonia. Therefore, more safe medications and treatments are in demand to prevent alcoholic disorders.

Contrary to synthetic medicine treatments, many Chinese traditional medicines have been known to have preventive and therapeutic effects for ethanol intoxication, but their active components have not been identified so far. Recently, we isolated a number of saponins with inhibitory effect on ethanol absorption from natural medicines. Some examples include elatosides from the bark of *Aralia elata*, sapindosides from the pericarps of *Sapindus mukurossi*, escins from the seeds of *Aesculus hippocastanum*, camelliasaponins from the seeds of *Camellia japonica*, senegins and senegasaponins from the root of *Polygala senega* var. *latifolia*, and momordins from the fruit of *Kochia scoparia*.[4–9] Furthermore, we

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0968-0896/00/$ - see front matter
PII: S0968-0896(00)00127-9

have characterized dihydroflavonols named hovenitins that have an inhibitory effect on alcohol-induced muscular relaxation and hepatoprotective activity from the fruit of *Hovenia dulcis*.[10]

In the course of our studies on anti-alcohol substances from natural resources, we found that the methanolic extract of the leaves of Lauraceae plant, *Laurus nobilis* (bay leaf, laurel) especially showed potent inhibitory activity on ethanol absorption in rats. This plant is widely cultivated in Mediterranean countries and the leaves are used as a herb in cooking for soup or stew as well as herbal medicine for excitant, stomachic, and digestant purposes in Europe. The pharmacological properties of *L. nobilis* include inhibitory effects on NO production (leaves), anti-ulcer (seeds) and anti-diabetic (leaves) effects, and the enhancement of liver glutathione *S*-transferase (GST) activity.[11–14] In this report, we describe the isolation of seven sesquiterpenes (**1**–**7**) from the leaves of *L. nobilis*, its inhibitory effect on ethanol absorption, and the elucidation of the structure requirements for its activity.[15] In addition, we describe the acute toxicity level of the principal inhibitors, costunolide (**1**) and dehydrocostus lactone (**2**), comparing to the active compound, α-methylene-γ-butyrolactone (**12**).

Results and Discussion

Effects of extract and sesquiterpenes isolated from *L. nobilis* on blood ethanol elevation in ethanol-loaded rats

Table 1 shows the effects of the extract and fractions obtained from the leaves of *L. nobilis* on blood ethanol elevation in ethanol-loaded (20%, 5 mL/kg) rats. Methanolic extract (yield: 20.0% from dried leaves) inhibited the elevation of blood ethanol level from 125 to 500 mg/kg in a dose-dependent manner. The ethyl acetate soluble fraction (7.3%) also strongly inhibited blood ethanol elevation, but no suppressive effect was observed in the water soluble fraction (12.7%). Then, we performed bioassay-guided separation of the ethyl acetate soluble fraction and isolated four types of sesquiterpenes, that is, germacrane-type (costunolide[16] (**1**, 0.18%)), guiane-type (dehydrocostus lactone[17] (**2**, 0.008%) and zaluzanin D[18] (**3**, 0.006%)), eudesmane-type (reynosin[19] (**4**, 0.018%), santamarine[20] (**5**, 0.041%), 3α-acetoxyeudesma-1,4(15),11(13)-trien-12,6α-olide[21] (**6**, 0.004%), 3-oxoeudesma-1,4,11(13)-trien-12,6α-olide[22] (**7**, 0.009%) and β-eudesmol[23] (**8**, 0.0025%)), and caryophyllene-type (β-caryophyllene[24] (**9**, 0.0008%) and caryophyllene oxide[25] (**10**, 0.0008%)) (Chart 1). Fig. 1 shows the effects of the sesquiterpenes on blood ethanol elevation. Compounds **1**–**7** having an α-methylene-γ-butyrolactone moiety inhibited blood ethanol elevation at 50 mg/kg. In particular, **1**, **6** and **7** showed potent suppression at 25 mg/kg. On the other hand, **8**–**10** lacking the lactone moiety had no effect at 50 mg/kg. These results suggest that an α-methylene-γ-butyrolactone moiety is needed to inhibit blood ethanol elevation.

Table 1. Effects of methanolic extract and its fractions from the leaves of *Laurus nobilis* on blood ethanol elevation in ethanol-loaded rats[a]

Treatment	Dose (mg/kg)	*N*	Blood ethanol (mg/mL) 0.5 h	1 h	2 h
Control	—	5	0.64±0.04	0.59±0.03	0.28±0.04
Methanolic extract	125	5	0.15±0.04**[b]	0.34±0.10**	0.32±0.03
	250	5	0.05±0.02**	0.05±0.02**	0.11±0.03**
	500	5	0.01±0.01**	0.01±0.01**	0.03±0.02**
Control	—	5	0.88±0.03	0.64±0.02	0.32±0.02
AcOEt-soluble fraction	125	5	0.12±0.02**	0.24±0.06**	0.10±0.05**
	250	5	0.05±0.01**	0.08±0.01**	0.11±0.04**
Water-soluble fraction	125	5	0.90±0.04	0.65±0.03	0.34±0.02
	250	5	0.87±0.03	0.64±0.04	0.36±0.04

[a]Each compound was given orally to fasted (20–22 h) rats and ethanol (20%, 5 mL/kg) was loaded 30 min thereafter. Blood samples were collected from the infraorbital venous plexus at 0.5, 1 and 2 h after administration of ethanol. Values are means±S.E.M.
[b]Asterisks denote significant differences from control, **: $P < 0.01$.

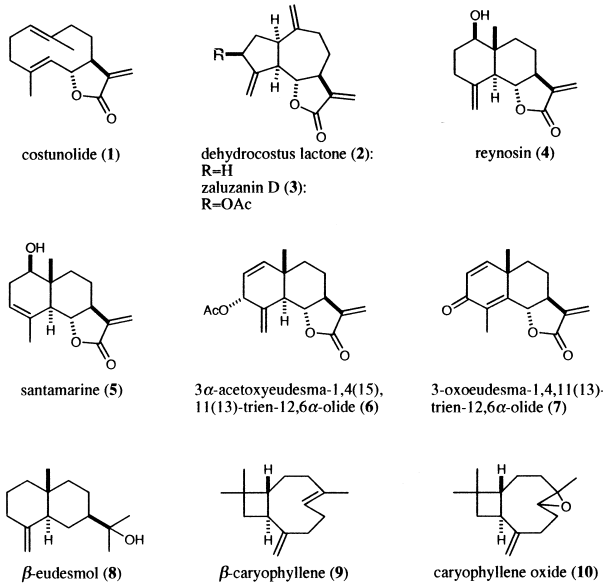

Chart 1. Sesquiterpenes (**1**–**10**) isolated from the leaves of *Laurus nobilis*.

Synthesis of reduction products (2a–2d) and amino acid adducts (2e, 2f) from dehydrocostus lactone (2)

To study the structure–activity relationships in the α-methylene-γ-butyrolactone moiety of **2**, we prepared four reductants (**2a**–**2d**) and two amino acid adducts (**2e**, **2f**)[26,27] linked with L-proline and L-cysteine at the 13-position in **2** (Chart 2). The reductants (**2a**–**2d**) were prepared by the following procedure: namely, the treatment of **2** with lithium aluminum hydride ($LiAlH_4$) gave a 11α-methyl-6,12-diol derivative[28] (**2a**, 46%), and treatment with sodium borohydride ($NaBH_4$) yielded mokko lactone[28] (**2b**, 18%), the 11α-methyl lactol mixture[28] (**2c**, 70% ca. 1:1 ratio), and the 11α-methyl-12-methylene derivative (**2d**, 9%). These known constituents (**2a**, **2b** and **2c**) were identified by comparison of their physical data with reported values.[28]

The 11α-methyl-12-methylene derivative (**2d**) showed a molecular ion (M^+) peak at *m*/*z* 218 in the electron

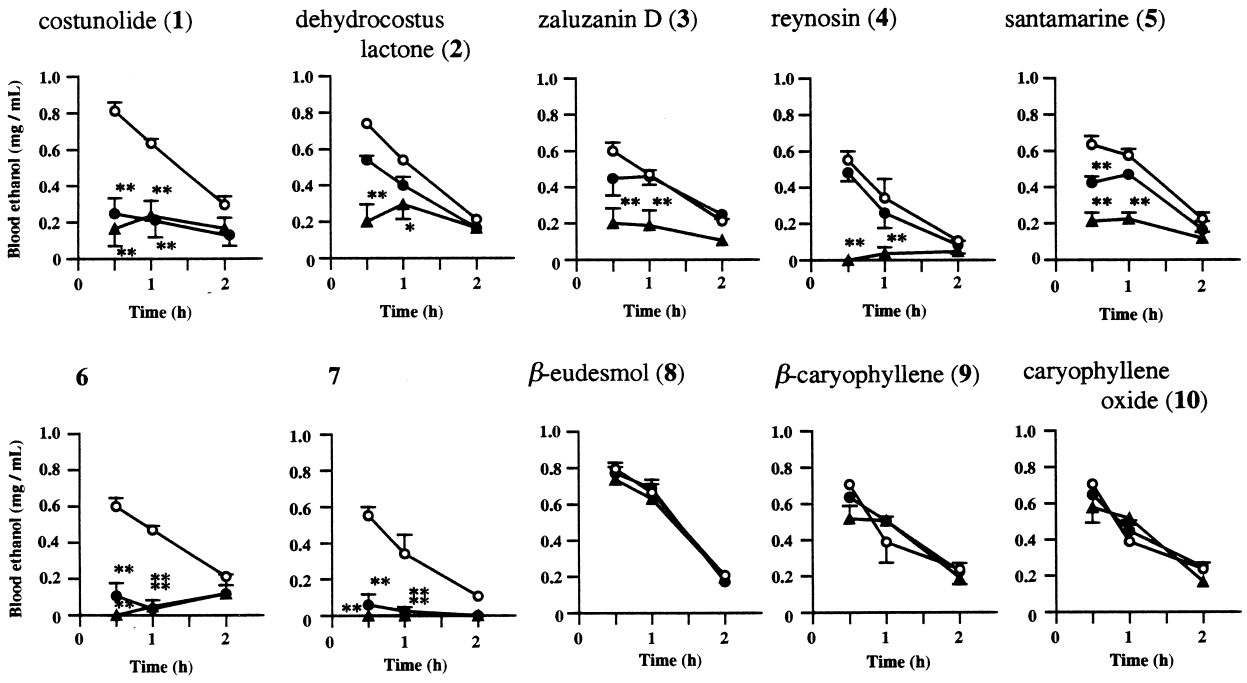

Figure 1. Effects of the sesquiterpenes from the leaves of *L. nobilis* on blood ethanol elevation in ethanol-loaded rats. Each compound was given orally to fasted (20–22 h) rats, and ethanol (20%, 5 mL/kg) was loaded 30 min thereafter. Blood samples were collected from the infraorbital venous plexus at 0.5, 1 and 2 h after administration of ethanol. Symbols are represented by the following , control: ○, 25 mg/kg: ●, and 50 mg/kg: ▲. Values are means with S.E.M. ($N = 3$–5), and asterisks denote significant differences from control, *: $P < 0.05$, **: $P < 0.01$.

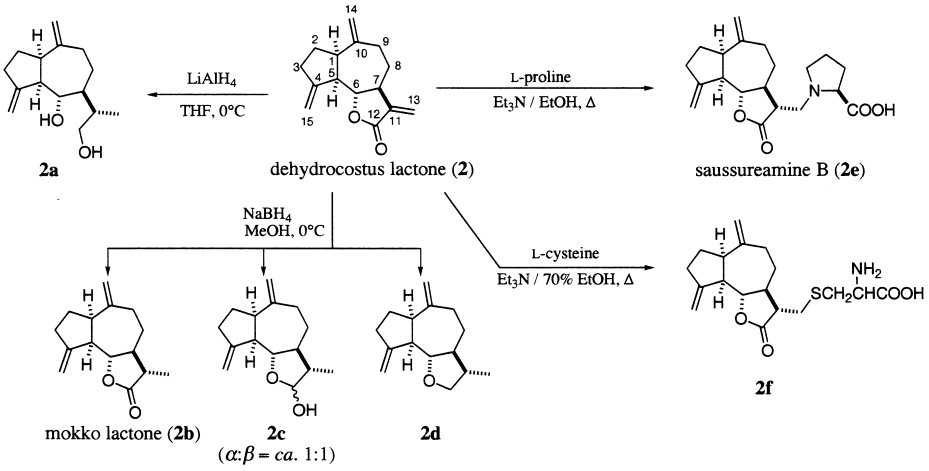

Chart 2. Reduction products (**2a**–**2d**) and amino acid adducts (**2e**, **2f**) from dehydrocostus lactone (**2**).

impact (EI)-MS measurement, and its molecular formula $C_{15}H_{22}O$ was determined by high-resolution MS measurement. The IR spectrum of **2d** showed no absorption bands due to the γ-lactone function, which was observed in that of **2**. The ^{1}H NMR ($CDCl_3$) and ^{13}C NMR (Table 2) spectra of **2d** were closely similar to those of **2b**, except for the signals due to the 12-methylene (δ 3.48 (1H, dd, $J = 6.1$, 11.0 Hz, 12α-H), 3.66 (1H, dd, $J = 5.2$, 11.0 Hz, 12β-H)) function. Furthermore, the stereostructure of the 11-position of **2d** was determined by the nuclear Overhauser effect spectroscopy (NOESY) experiment, in which the NOE correlation was observed between the 6-proton (δ 3.23 (1H, dd, $J = 8.5$, 8.6 Hz)) and the 11-proton (δ 2.09 (1H, dddq, $J = 5.2$, 6.1, 10.3, 7.0 Hz)). Thus, the stereostructure of **2d** was characterized to be the hydrofuran structure having a 11α-methyl group.

Amino acid adducts (**2e**, **2f**) were prepared in a similar manner as described in previous papers.[26,27] Saussureamine B (**2e**) and **2f** were obtained by treatment of **2** with L-proline or L-cysteine in the presence of triethylamine in ethanol or 70% aqueous ethanol.

Effects of synthetic derivatives from 2 on blood ethanol elevation in ethanol-loaded rats

Table 3 shows the inhibitory activity of the derivatives (**2a**–**2f**) from **2** on ethanol absorption. The diol derivative (**2a**) completely lacked the activity which shows that the lactone ring is necessary for the inhibitory activity. Mokko lactone (**2b**) (a reductant of the 11-*exo*-methylene group) and **2c** (a reductant of the 12-carbonyl group) maintained the activity. On the other hand, compound

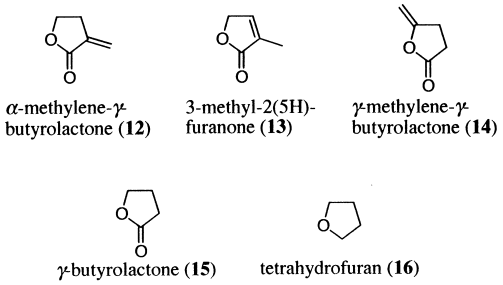

Chart 3. Chemical structures of γ-butyrolactones and a related compound.

Table 2. ^{13}C NMR data for **2c** and **2d**[a]

	2c		2d
	12α-OH	12β-OH	
C-1	47.6	47.6	46.2
C-2	30.0	29.9	29.0
C-3	32.4*[b]	32.1*	31.2
C-4	153.8	153.2	154.2
C-5	54.6	52.7	54.1
C-6	86.7	83.7	68.4
C-7	47.8	53.3	47.1
C-8	31.6	32.2	26.9
C-9	38.0	37.6	34.3
C-10	151.4**	151.1**	151.6
C-11	45.3	49.2	36.3
C-12	99.3	104.0	66.5
C-13	11.4	15.8	13.5
C-14	110.8	110.8	109.9
C-15	108.1***	107.7***	110.8

[a]Measured in $CDCl_3$ (δ) at 125 MHz.
[b]*, **, ***Assignments may be interchangeable in same row.

2d lacking the 12-oxygen function, and amino acid adducts (**2e**, **2f**) lacked the activity. These results indicate that a lactone or a lactol ring is essential for the activity. It is not yet clarified that whether the *exo*-methylene or the methyl group participate in the expression of the activity, because both **2** and **2b** showed the activity. However, since amino acid adducts (**2e**, **2f**) lacked the activity, a large hydrophilic group such as amino acid groups conjugated to the methyl group at the 13-position seems to reduce the activity.

Effects of the γ-lactone derivatives (12–15) and tetrahydrofuran (16) on blood ethanol elevation in ethanol-loaded rats

Table 4 shows the activities of the γ-lactone derivatives (**12**–**15**) and tetrahydrofuran (**16**) which are suggested to be the active site of the sesquiterpenes (**1**–**7**) from *L. nobilis*. α-Methylene-γ-butyrolactone (**12**) dramatically suppressed blood ethanol elevation at doses of 12.5 to 50 mg/kg. 3-Methyl-2(5H)-furanone (**13**) also suppressed blood ethanol elevation at doses of 25 and 50 mg/kg. These results correlate with results of **2** and **2b**. γ-Butyrolactone (**15**) and tetrahydrofuran (**16**) had no activity. In addition, γ-methylene-γ-butyrolactone (**14**), an isomer of **12**, lacked the activity at 50 mg/kg. These results indicate that the lactone ring with an α-methylene or α-methyl group in the ring is essential for the activity (Chart 3).

Effects of the costunolide (1), dehydrocostus lactone (2), and α-methylene-γ-butyrolactone (12) on serum glucose elevation in glucose-loaded rats

In order to study the selectivity of the active sesquiterpenes on blood ethanol elevation, we investigated the effects of **1**, **2** and **12** on glucose absorption in rats. Figure

Table 3. Effects of derivatives (**2a**–**2f**) of dehydrocostus lactone (**2**) on blood ethanol elevation in ethanol-loaded rats[a]

	Dose (mg/kg)	N	Blood ethanol (mg/mL) 0.5 h	1 h	2 h
Control	—	5	0.74±0.02	0.54±0.01	0.21±0.01
2	25	5	0.55±0.03	0.40±0.05	0.17±0.02
	50	5	0.20±0.09**[b]	0.29±0.09*	0.17±0.02
Control	—	5	0.78±0.04	0.75±0.07	0.25±0.02
2a	50	5	0.80±0.04	0.66±0.06	0.28±0.02
2c	25	5	0.68±0.07	0.60±0.06	0.17±0.05
	50	5	0.24±0.04**	0.31±0.10**	0.31±0.07
Control	—	5	0.67±0.04	0.65±0.07	0.22±0.02
Mokko lactone (**2b**)	25	5	0.14±0.04**	0.33±0.09	0.17±0.05
	50	5	0.04±0.01**	0.41±0.15	0.21±0.08
Control	—	4	0.61±0.06	0.45±0.04	0.17±0.04
2d	50	4	0.47±0.09	0.40±0.09	0.18±0.03
Control	—	5	0.82±0.04	0.64±0.04	0.37±0.04
Saussureamine B (**2e**)	50	4	0.76±0.07	0.59±0.03	0.36±0.04
2f	50	4	0.85±0.02	0.60±0.03	0.32±0.10

[a]Values are means±S.E.M.
[b]Asterisks denote significant differences from control, *: $P < 0.05$, **: $P < 0.01$.

Table 4. Effects of γ-butyrolactone derivatives (**12**–**16**) on blood ethanol elevation in ethanol-loaded rats[a]

	Dose (mg/kg)	N	Blood ethanol (mg/mL) 0.5 h	1 h	2 h
Control	—	5	0.68±0.02	0.61±0.05	0.14±0.04
α-Methylene-γ-butyrolactone (**12**)	12.5	5	0.32±0.04**[b]	0.71±0.05	0.38±0.01
	25	5	0.07±0.04**	0.18±0.10**	0.03±0.03
	50	5	0.04±0.02**	0.12±0.07**	0.04±0.03
Control	—	5	0.82±0.04	0.64±0.04	0.37±0.04
3-Methyl-2(5H)-furanone (**13**)	25	4	0.26±0.07**	0.28±0.10*	0.30±0.07
	50	4	0.10±0.04**	0.34±0.15	0.16±0.11
Control	—	4	0.54±0.07	0.51±0.05	0.18±0.04
γ-Methylene-γ-butyrolactone (**14**)	25	4	0.59±0.06	0.60±0.06	0.17±0.05
	50	4	0.67±0.02	0.53±0.01	0.12±0.07
Control	—	5	0.42±0.03	0.42±0.03	0.17±0.03
γ-Butyrolactone (**15**)	25	5	0.42±0.05	0.34±0.06	0.16±0.03
	50	5	0.51±0.04	0.35±0.03	0.12±0.04
Control	—	6	0.80±0.05	0.72±0.03	0.48±0.15
Tetrahydrofuran (**16**)	25	5	0.82±0.03	0.73±0.03	0.33±0.02
	50	4	0.74±0.07	0.67±0.02	0.34±0.01

[a]Values are means±S.E.M.
[b]Asterisks denote significant differences from control, *: $P < 0.05$, **: $P < 0.01$.

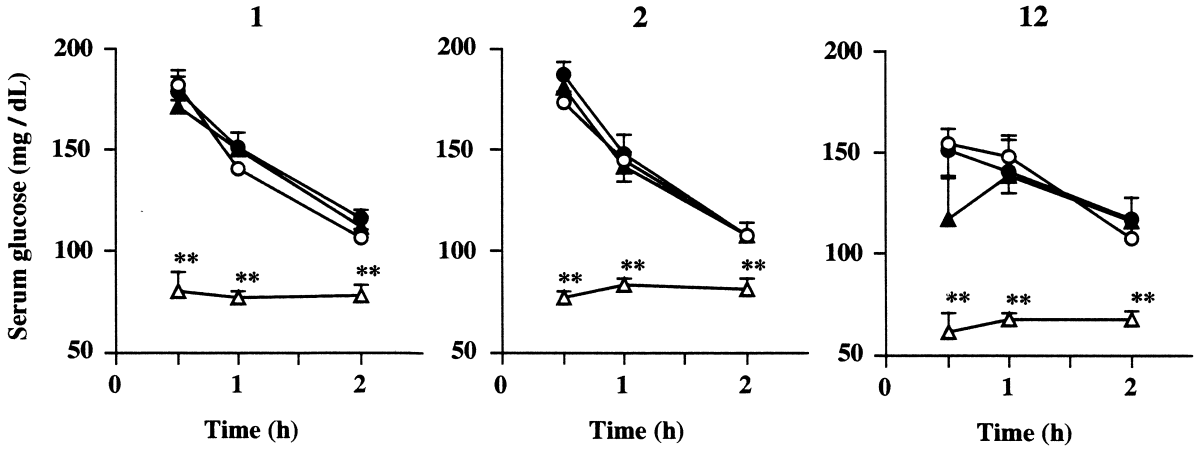

Figure 2. Effects of costunolide (**1**), dehydrocostus lactone (**2**), and α-methylene-γ-butyrolactone (**12**) on increase of serum glucose level in glucose-loaded rats. Each compound was given orally to fasted (20–22 h) rats and glucose (1 g/kg) was loaded 30 min thereafter. Blood samples were collected from the infraorbital venous plexus at 0.5, 1, and 2 h after administration of glucose. Symbols are represented by the following, control: ○, 25 mg/kg: ●, 50 mg/kg: ▲, and glucose untreated group △. Values are means with S.E.M. ($N = 5$), and asterisks denote significant differences from control *: $P < 0.05$, **: $P < 0.01$.

2 shows the effects of the **1**, **2** and **12** on serum glucose elevation in glucose-loaded (1 g/kg) rats. Costunolide (**1**) and dehydrocostus lactone (**2**) have no influence on serum glucose level at 50 mg/kg. Compound **12** slightly suppressed serum glucose elevation at 50 mg/kg, but the effect was not significant. These results suggest that sesquiterpenes from *L. nobilis* selectively inhibit the alcohol absorption rather than the glucose absorption.

Single dose oral toxicity of costunolide (1), dehydrocostus lactone (2) and α-methylene-γ-butyrolactone (12) in mice

Table 5 shows the result of single dose oral toxicities of **1**, **2** and **12** in mice. No death was observed in the groups given **1** or **2** at doses of 250 and 500 mg/kg. On the other hand, all mice given **12** were dead within 24 h (250 mg/kg) and 1 h (500 mg/kg). The mice in the group given **12** at 500 mg/kg died of breathing cessation and generalized convulsion. γ-Butyrolactone (**15**) is reported to induce an increase in L-dihydroxyphenylalanine (DOPA) synthesis and its accumulation in the brain.[29,30] Its metabolite, γ-hydroxybutyric acid, easily passes through the brain barrier and causes DOPA accumulation in the striatum.[31] DOPA accumulation leads to a decrease in dopamine synthesis and causes reduction of dopamine neuronal impulse flow.[32,33] Since the chemical structure of **12** is similar to **15**, **12** may act on the brain and show toxicity by the damage of neurotransmission through dopamine.

In conclusion, we isolated seven active sesquiterpenes (**1**–**7**) from the leaves of *L. nobilis* as alcohol absorption inhibitors. The active moiety in these sesquiterpenes was found to be the α-methylene-γ-butyrolactone moiety. Furthermore, a lactone or a lactol ring with an α-methyl or α-methylene group was essential to show suppression of ethanol absorption. Alternative medical treatments such as these can be used to regulate alcohol ingestion or absorption and reduce the risk of alcoholic disorders. These sesquiterpenes not only have a potent preventive effect for acute alcohol toxicity but also can be available to help patients recover from addiction to alcohol abuse.

Table 5. Single dose oral toxicity of costunolide (**1**), dehydrocostus lactone (**2**), and α-methylene-γ-butyrolactone (**12**) in mice[a]

			Mortality					
	Dose (mg/kg)	*N*	1	3	6	24 (h)	7 (day)	Total
1	250	6	0	0	0	0	0	0
	500	6	0	0	0	0	0	0
2	250	6	0	0	0	0	0	0
	500	6	0	0	0	0	0	0
12	50	6	0	0	0	0	0	0
	125	6	0	0	0	0	0	0
	250	6	1	2	0	3	—	6
	500	6	6	—	—	—	—	6

[a]Each compound was given orally to fasted (22 h) mice. Mice were continuously fasted for 24 h and housed in standard conditions for 7 days.

Experimental

The following instruments were used to measure physical data of dehydrocostus lactone (**2**) derivatives: specific rotations, Horiba SEPA-300 digital polarimeter ($l = 5$ cm); IR spectra, Shimadzu FTIR-8100 spectrometer; EI-MS and high-resolution MS, JEOL JMS-GCMATE mass spectrometer; FAB MS and high-resolution MS, JEOL JMS-SX 102A mass spectrometer; ^{1}H NMR spectra, JNM-LA500 (500 MHz) spectrometer; ^{13}C NMR spectra, JNM-LA500 (125 MHz) spectrometer with tetramethylsilane as an internal standard.

The following experimental conditions were used for chromatography: ordinary-phase silica gel column chromatography, Silica-gel BW-200 (Fuji Silysia Chemical, Ltd, 150–350 mesh); reversed-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd, 100–200 mesh); TLC, pre-coated TLC plates with Silica gel $60F_{254}$ (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 $60F_{254}$ (Merck, 0.25 mm) (reversed-phase); reversed-phase HPTLC, pre-coated TLC plates with Silica gel RP-18 $60WF_{254S}$ (Merck, 0.25 mm); detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4 and heating.

Animals and materials

Male Wistar rats aged 4–6 weeks and ddY mice aged 5 weeks were purchased from Kiwa Laboratory Animals Co., Ltd (Wakayama, Japan), and housed in an air-conditioned room at 23±2 °C. Standard laboratory chow (MF, Oriental Yeast Co., Ltd, Tokyo, Japan) and tap water were given ad libitum. They were fasted for 20–22 h prior to experiments, but were supplied with water ad libitum. Test samples were suspended in 5% acacia solution and given orally at 5 mL/kg in each experiment.

γ-Butyrolactones (**12**, **14**, **15**) were purchased from Sigma-Aldrich Chemie Gmbh (Steinheim, Germany). 3-Methyl-2(5H)-furanone (**13**) and tetrahydrofuran (**16**) were obtained from Tokyo Kasei Kogyo Co. Ltd (Tokyo, Japan).

Isolation of chemical constituents from the leaves of *L. nobilis*

The dried leaves of *L. nobilis* (5.0 kg, harvested in Turkey) were extracted with methanol at room temperature and evaporated in vacuo under 40 °C to obtain the methanolic extract (999 g, 20.0% from the leaves). The methanolic extract (366 g) was partitioned into an ethyl acetate–water mixture to obtain the ethyl acetate-soluble fraction (134 g, 7.3%) and the water-soluble fraction (232 g, 12.7%). The ethyl acetate-soluble fraction (130 g) was subjected to ordinary-phase silica-gel chromatography (2.6 kg, hexane:AcOEt (30:1)→AcOEt→MeOH) to give eleven fractions (Fr.1 (747 mg), Fr.2 (7.55 g), Fr.3 (7.33 g), Fr.4 (23.3 g), Fr.5 (3.48 g), Fr.6 (13.0 g), Fr.7 (3.76 g), Fr.8 (10.5 g), Fr.9 (11.7 g), Fr.10 (10.2 g), Fr.11 (35.2 g)). Fraction 1 (747 mg) was separated by ordinary-phase silica-gel [35 g, hexane:AcOEt (50:1)→(3:1)) to give β-caryophyllene (**9**, 14 mg, 0.0008%) and caryophyllene oxide (**10,** 14 mg, 0.0008%). A part of each fraction 4–8 was subjected to reversed-phase silica-gel (60% aq MeOH→100% MeOH) column chromatography and finally HPLC (Cosmosil $5C_{18}$, 250×20 mm i.d., 50%→80% aq MeOH) to give costunolide (**1**, 767 mg, 0.18%), dehydrocostus lactone (**2**, 34 mg, 0.008%), zaluzanin D (**3**, 96 mg, 0.006%), reynosin (**4**, 299 mg, 0.018%), santamarine (**5**, 447 mg, 0.041%), 3α-acetoxyeudesma-1,4(15),11(13)-trien-12,6α-olide (**6**, 70 mg, 0.004%), 3-oxoeudesma-1,4,11(13)-trien-12,6α-olide (**7**, 155 mg, 0.009%), and β-eudesmol (**8**, 42 mg, 0.0025%). These sesquiterpenes (**1**–**10**) were identified by comparison of their physical data with those of authentic samples (**1**, **2**, **5**) or with reported values.[18,19,21–25]

Measurement of blood ethanol elevation in ethanol-loaded rats

Test samples were administered orally to fasted (20–22 h) rats. Thirty min thereafter, ethanol (20 v/v%, 5 mL/kg) was given orally. Blood samples were collected from the infra-orbital venous plexus at 0.5, 1 and 2 h after the administration of ethanol. Then blood was immediately mixed with 10-fold volume of ice-cold 0.33N perchloric acid and centrifuged (4 °C, 3000 r.p.m., 10 min). Blood ethanol level in the supernatant was assessed by the enzymatic method (F-kit® ethanol, Boehringer, Mannheim, Germany).

Chemical transformation of dehydrocostus lactone (2)

Reduction of 2 with $LiAlH_4$. A solution of **2** (150 mg, 0.65 mmol) in tetrahydrofuran (THF, 5.0 mL) was treated with $LiAlH_4$ (62 mg) and the mixture was stirred at 0 °C for 30 min. The reaction mixture was then quenched with aq sat. ether and subsequently 4N aq KOH, and the whole was extracted with AcOEt. The AcOEt extract was washed with 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 HPLC (YMC-Pack ODS-A 250×20 mm i.d. (MeOH:H_2O 70:30 v/v)) to give **2a**[27] (66 mg, 46%).

Reduction of 2 with $NaBH_4$. A solution of **2** (600 mg, 2.6 mmol) in MeOH (10.0 mL) was treated with sodium borohydride ($NaBH_4$, 100 mg) and the mixture was stirred at 0 °C for 1 h. The reaction mixture was quenched at acetone, and extracted with AcOEt. The AcOEt extract was washed with 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 HPLC (YMC-Pack ODS-A 250×20 mm i.d. (MeOH:H_2O 75:25 v/v)) to give mokko lactone[28] (**2b**, 106 mg, 18%), two lactol mixture[28] (**2c**, 489 mg, 70%), and **2d** (53 mg, 9%).

2c. ^{1}H NMR ($CDCl_3$) δ: (the signals due to the 12α-OH derivative[34]) 1.03 (3H, d, J=6.7 Hz, 13-H_3), 1.10 (1H, m, 8β-H), 1.73 (1H, m, 11-H), 1.82, 1.92 (1H each, both m, 2-H_2), 1.90 (1H, m, 7-H), 2.03 (1H, m, 8α-H), 2.06, 2.45 (1H each, both m, 9-H_2), 2.48, 2.50 (1H each, both m, 3-H_2), 2.74 (1H, dd, J=8.8, 9.2 Hz, 5-H), 2.83 (1H, m, 1-H), 3.54 (1H, dd, J=9.2, 9.5 Hz, 6-H), 4.70, 4.81 (1H each, both s, 14-H_2), 4.99, 5.15 (1H each, both br s, 15-H_2), 5.25 (1H, dd, J=4.0, 4.3 Hz, 12β-H) (the signals due to 12β-OH derivative[34]) 1.10 (3H, d, J=7.0 Hz, 13-H_3), 1.23 (1H, m, 8β-H), 1.56 (1H, m, 7-H), 1.69 (1H, m, 11-H), 1.82, 1.92 (1H each, both m, 2-H_2), 1.97, 2.43 (1H each, both m, 9-H_2), 2.00 (1H, m, 8α-H), 2.48, 2.50 (1H each, both m, 3-H_2), 2.67 (1H, dd, J=8.5, 9.4 Hz, 5-H), 2.81 (1H, m, 1-H), 3.74 (1H, dd, J=9.4, 9.5 Hz, 6-H), 4.68, 4.81 (1H each, both s, 14-H_2), 4.99, 5.15 (1H each, both br s, 15-H_2), 5.06 (1H, dd, J=4.3, 4.8 Hz, 12α-H). ^{13}C NMR ($CDCl_3$): given in Table 2.

2d. colorless oil, $[\alpha]_D^{25}$ +44.8° (c=0.1, $CHCl_3$). High-resolution EI–MS: Calcd for $C_{15}H_{22}O$ (M^+): 218.1671. Found : 218.1668. IR (film): 1028, 887 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 0.91 (3H, d, J=7.0 Hz, 13-H_3), 1.39 (1H, dddd, J=4.5, 8.9, 12.5, 17.7 Hz, 8β-H), 1.66 (1H, dddd, J=1.8, 4.5, 8.6, 10.3 Hz, 7-H), 1.81 (1H, dddd, J=3.7, 8.9, 12.9, 17.5 Hz, 2-H), 1.90 (1H, dddd, J=1.8, 4.0, 8.5, 17.7 Hz, 8α-H), 1.99 (1H, dddd, J=5.5, 7.9, 13.4, 17.5 Hz, 2-H), 2.09 (1H, dddq, J=5.2, 6.1, 10.3, 7.0 Hz, 11-H), 2.09, 2.35 (1H each, both m, 9-H_2), 2.33, 2.54 (1H each, both m, 3-H_2), 2.76 (1H, dd, J=8.0, 8.5 Hz, 5-H), 2.90 (1H, ddd, J=7.9, 8.0, 8.9 Hz, 1-H), 3.23 (1H, dd, J=8.5, 8.6 Hz, 6-H), 3.48 (1H, dd, J=6.1, 11.0 Hz, 12α-H), 3.66 (1H, dd, J=5.2, 11.0 Hz, 12β-H), 4.72 (1H, dd, J=1.8, 1.9 Hz, 14-H), 4.84 (1H, d, J=1.2 Hz, 14-H), 5.03, 5.13 (1H each, both br s, 15-H_2). ^{13}C NMR ($CDCl_3$): given in Table 2. EI–MS m/z (%): 218 (M^+, 25), 159 (100).

Measurement of serum glucose elevation in glucose-loaded rats

Test samples were administered orally to fasted rats. Thirty min thereafter, glucose (1 g/kg) was given orally. Blood samples were collected from the infraorbital venous plexus at 0.5, 1, and 2 h after the administration of glucose. Then, blood was centrifuged (4 °C, 3000 r.p.m., 10 min). Serum glucose level was assessed by enzymatic method (Glucose C-II test Wako®, Wako Pure Chemical, Osaka, Japan).

Single dose oral toxicity in mice

Various doses of costunolide (**1**), dehydrocostus lactone (**2**), or α-methylene-γ-butyrolactone (**12**) were administered orally to 22 h-fasted mice. Mice were continuously fasted for 24 h and housed in standard conditions for 7 days. General symptoms and death in mice were observed during the experimental period.

Statistics

Values are expressed as means±S.E.M. Significant differences were calculated by Dunnett's multiple comparison test. Probability (P) values less than 0.05 were considered significant.

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