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Inhibitory Effect of 2-(*E*-2-Alkenoylamino)ethyl Alkyl Sulfides on Gastric Ulceration in Rats. I. Effect of 2-(*E*-2-Alkenoylamino)ethyl Carbamoylmethyl Sulfides on Gastric Secretion and Various Ulceration Models in Rats

MASAKAZU IWAI,*,[a] ISAO KOHDA,[a] CHIKARA FUKAYA,[a] YOICHIRO NAITO,[a] KAZUMASA YOKOYAMA,[a] HIROSHI NAKAJIMA,[b] KAZUTAKE TSUJIKAWA,[b] MASARU OKABE[b] and TSUTOMU MIMURA[b]

Central Research Laboratories, The Green Cross Corporation,[a] Miyakojima-ku, Osaka 534, Japan and Faculty of Pharmaceutical Sciences, Osaka University,[b] Yamadaoka 1–6, Suita, Osaka 565, Japan

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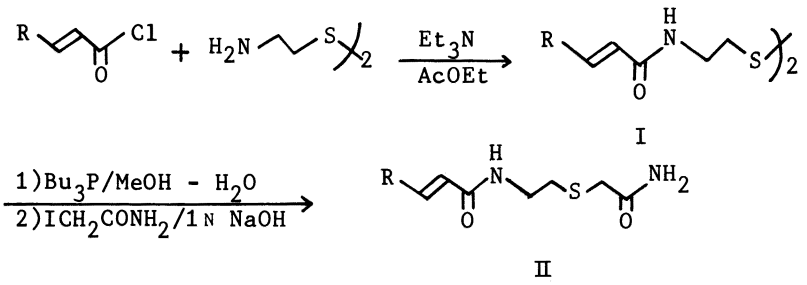
Bis[2-(*E*-2-alkenoylaminoethyl)] disulfides (I) and 2-(*E*-2-alkenoylamino)ethyl carbamoylmethyl sulfides (II) with various alkenyl chain lengths were synthesized and their inhibitory effects on gastric secretion in rats were compared. There was a relationship between the alkenyl chain length of a series of sulfide derivatives (II) and their biological activities (C10 and C11 alkenyl derivatives were the most effective compounds). On the other hand, variation of the alkenyl chain length did not affect the anti-ulcerogenic activity of the disulfide derivatives (I). The derivatives (II) showed stronger biological activity than (I) when the same alkenyl group was present in both.

The administration of 2-(*E*-2-decenoylamino)ethyl carbamoylmethyl sulfide (II-5) or 2-(*E*-2-undecenoylamino)ethyl carbamoylmethyl sulfide (II-6) at a dose of 20 mg/kg (i.p.) caused significant inhibition of various experimental ulcerations caused by stress, aspirin and HCl–ethanol. Oral administration of both acetamides (II-5, II-6) also caused 50—60% inhibition of ulceration in the water-immersion stress model at a dose of 20 mg/kg, although the activity was not as strong as that after i.p. injection. An improvement in anti-ulcerogenic activity was observed when acetamides were administered as a suspension in 10% HCO-60. Both acetamides (II-5, II-6) caused a dose-dependent decrease of the ulcer index of restrained and water-immersion stress-loaded rats in the dosage range from 0.5 mg/kg *p.o.* to 5 mg/kg *p.o.* The lethal dose 50% values (LD_{50}) for both acetamides were over 8 g/kg (*p.o.* or i.p.).

Keywords——bis[(2-(*E*-2-alkenoylaminoethyl)] disulfide; 2-(*E*-2-alkenoylamino)ethyl carbamoylmethyl sulfide; anti-ulcerogenic activity; antisecretory activity

We have reported that human immunoglobulin G (IgG) showed anti-ulcerogenic[1] and anti-inflammatory[2] activity after reductive cleavage of interchain disulfide bonds, though the native IgG showed no such activity. It was suggested that the chemical modification of S–S bonds in the hinge region was essential. On the other hand, we have reported the anti-ulcerogenic activity of various saturated[3] and unsaturated[4] fatty acids and investigated the relationship between alkyl or alkenyl chain lengths and biological activities.

In the present article, we describe our primary results in attempting to develop a new anti-ulcerogenic drug. As a first step, commercially available sulfur-containing amino acids and amines were chosen and their gastric secretion-inhibitory activity in pylorus-ligated rats were observed. Among the chemicals tested, cystamine showed the strongest activity. Therefore, alkenyl amino derivatives (I) were synthesized from various *trans*-2-unsaturated fatty acids and cystamine, and their pharmacological activities were tested together with those of the 2-(*E*-2-alkenoylamino)ethyl carbamoylmethyl sulfides (II) which were obtained by reduction and alkylation of the derivatives (I).



bis[2-(*E*-2-alkenoylaminoethyl)]disulfides (I)

I-1: R=C_3H_7 I-2: R=C_4H_9 I-3: R=C_5H_{11} I-4: R=C_6H_{13} I-5: R=C_7H_{15}
I-6: R=C_8H_{17} I-7: R=C_9H_{19} I-8: R=$C_{10}H_{21}$ I-9: R=$C_{11}H_{23}$

2-(*E*-2-alkenoylamino)ethyl carbamoylmethyl sulfides (II)

II-1: R=C_3H_7 II-2: R=C_4H_9 II-3: R=C_5H_{11} II-4: R=C_6H_{13} II-5: R=C_7H_{15}
II-6: R=C_8H_{17} II-7: R=C_9H_{19} II-8: R=$C_{10}H_{21}$ II-9: R=$C_{11}H_{23}$

Chart 1

Experimental

Reagents——Cystine, cysteine, methionine, ethionine, taurine, hypotaurine, methionine sulfone, cystamine dihydrochloride and cysteamine hydrochloride were from Nakarai Chemicals Ltd., Japan. Lanthionine and methionine sulfate were obtained from Tokyo Kasei Co., Japan. Test samples were suspended in 5% gum arabic in saline and were administered intraperitoneally at doses of 5, 10, and 25 mg/kg to the test animals.

Synthesis of Bis[(2-(*E*-2-alkenoylaminoethyl)] Disulfide Derivatives (I)——The following nine unsaturated fatty acids, containing one *trans* double bond between carbon numbers 2 and 3, were obtained from Tokyo Kasei Co., Japan: *trans*-2-hexenoic acid (C6 : 1), *trans*-2-heptenoic acid (C7 : 1), *trans*-2-octenoic acid (C8 : 1), *trans*-2-nonenoic acid (C9 : 1), *trans*-2-decenoic acid (C10 : 1), *trans*-2-undecenoic acid (C11 : 1), *trans*-2-dodecenoic acid (C12 : 1), *trans*-2-tridecenoic acid (C13 : 1) and *trans*-2-tetradecenoic acid (C14 : 1).

We synthesized the desired bis[(2-(*E*-2-alkenoylaminoethyl)] disulfides (I) from cystamine and the nine 2-*trans*-unsaturated fatty acids according to the method shown in Chart 1. First, the unsaturated fatty acid halides, obtained by reaction between the unsaturated fatty acids and thionyl chloride, were reacted with cystamine to give (I). After reduction of the disulfide bond in cystamine with tributylphosphine, the generated SH groups were alkylated with iodoacetamide to afford 2-(*E*-2-alkenoylamino)ethyl carbamoylmethyl sulfides (II).

The synthetic procedures, infrared data and ^{1}H nuclear magnetic resonance (^{1}H-NMR) spectrum data for the 18 objective compounds are given below.

Synthesis of Bis[(2-(*E*-2-hexenoylaminoethyl)] Disulfide (I-1)——A mixture of *trans*-2-hexenoic acid (15 g) and thionyl chloride (20 ml) was refluxed for 15 h. After the reaction, excess thionyl chloride was distilled off and the residue was distilled under reduced pressure to afford the acid chloride [(11.3 g, 64%, b.p. 77—78 °C (34 mmHg)].

A solution of this acid chloride (9.6 g) in ethyl acetate (15 ml) was added dropwise to an ice-cooled solution of cystamine (5.0 g) and triethylamine (10.1 ml) in ethyl acetate (150 ml). The reaction was carried out for 30 min at 0 °C. The mixture was then left overnight at room temperature. Water (100 ml) was added, and the precipitated solid was collected by filtration. Compound I-1 was obtained by recrystallization from ethyl acetate (10.8 g, 57%). mp 137—138 °C (pale yellow crystals). IR ν_{max}^{KBr} cm^{-1}: 3300, 3050, 2950, 2860, 1665, 1620, 1540, 1465, 975. ^{1}H-NMR ($CDCl_3$) δ: 0.90 (t, 6H, J=7 Hz), 2.13 (dt, 4H, J=6, 6 Hz), 2.80 (t, 4H, J=6 Hz), 3.58 (dt, 4H, J=6, 6 Hz), 5.85 (dt, 2H, J=16, 1 Hz), 6.80 (dt, 2H, J=16, 16 Hz), 6.90 (br, 2H).

Synthesis of 2-(*E*-2-Hexenoylamino)ethyl Carbamoylmethyl Sulfide (II-1)——Compound I-1 (2.0 g) was suspended in 60 ml of a 9 : 1 (v/v) mixture of methanol and water, and then tributylphosphine (1.3 g) was added dropwise to the suspension under an N_2 gas atmosphere. The reaction was carried out for 30 min at 0 °C and the mixture was allowed to stand for an additional hour at room temperature. Iodoacetamide (2.3 g) was then added, and 1 N NaOH (12.2 ml) was added dropwise to the cooled mixture. The whole was stirred for 1 h at room temperature. Water (100 ml) was added, and the white precipitate was collected by filtration. After recrystallization from ethanol-water, II-1 was obtained (1.8 g, 67%). mp 143—144.5 °C (white crystals). IR ν_{max}^{KBr} cm^{-1}: 3380, 3300, 3180, 2950, 2860, 1645, 1620, 1542, 1460, 975. ^{1}H-NMR ($CDCl_3$ + CF_3COOH) δ: 0.94 (t, 3H, J=7 Hz), 2.28 (dt, 2H, J=6, 6 Hz), 2.88 (t, 2H, J=7 Hz), 3.45 (s, 2H), 3.60 (t, 2H, J=7 Hz), 6.00 (d, 1H, J=15 Hz), 7.08 (dt, 1H, J=15, 6 Hz), 7.0—8.60 (br, m, 3H).

Synthesis of Bis[(2-(*E*-2-heptenoylaminoethyl)] Disulfide (I-2)——The procedures used for I-1 were repeated with *trans*-2-heptenoic acid (15 g) to obtain I-2 (12.3 g, 65%). mp 124—125 °C (white crystals). IR ν_{max}^{KBr} cm^{-1}: 3300, 3050, 2950, 2850, 1665, 1620, 1540, 1465, 978. ^{1}H-NMR ($CDCl_3$) δ: 0.90 (br, 6H), 2.20 (br, 4H), 2.85 (t, 4H, J=7 Hz), 3.65

(dt, 4H, $J=7$, 7 Hz), 5.89 (dt, 2H, $J=15$, 1 Hz), 6.86 (dt, 2H, $J=15$, 6 Hz), 6.80 (br, 2H).

Synthesis of 2-(*E*-2-Heptenoylamino)ethyl Carbamoylmethyl Sulfide (II-2)——The procedures used for II-1 were repeated with I-2 (2.0 g) to obtain II-2 (1.5 g, 57%). mp 143—144 °C (white crystals). IR ν_{max}^{KBr} cm^{-1}: 3380, 3300, 3180, 2950, 2850, 1645, 1620, 1540, 1460, 975. ^{1}H-NMR (DMSO-d_6+$CDCl_3$) δ: 0.90 (br t, 3H, $J=6$ Hz), 2.20 (br, 2H), 2.67 (t, 2H, $J=7$ Hz), 3.10 (s, 2H), 3.30 (dt, 2H, $J=7$, 7 Hz), 5.83 (d, 1H, $J=15$ Hz), 6.65 (dt, 1H, $J=15$, 6 Hz), 6.90 (br, 1H), 7.30 (br, 1H), 7.90 (br t, 1H, $J=7$ Hz).

Synthesis of Bis[(2-(*E*-2-octenoylaminoethyl)] Disulfide (I-3)——The procedures used for I-1 were repeated with *trans*-2-octenoic acid (3.9 g) to obtain I-3 (3.4 g, 85%). mp 131—133 °C (white crystals). IR ν_{max}^{KBr} cm^{-1}: 3300, 3050, 2950, 2850, 1665, 1620, 1545, 975. ^{1}H-NMR ($CDCl_3$+CF_3COOH) δ: 0.90 (br t, 6H, $J=6$ Hz), 2.15 (br, 4H), 2.85 (t, 4H, $J=7$ Hz), 3.60 (dt, 4H, $J=7$, 7 Hz), 5.90 (dt, 2H, $J=16$, 1 Hz), 6.80 (dt, 2H, $J=16$, 7 Hz), 6.90 (br, 2H).

Synthesis of 2-(*E*-2-Octenoylamino)ethyl Carbamoylmethyl Sulfide (II-3)——The procedures used for II-1 were repeated with I-3 (1.0 g) to obtain II-3 (1.0 g, 77%). mp 146.5—147 °C (needle-shaped white crystals). IR ν_{max}^{KBr} cm^{-1}: 3370, 3300, 3170, 2920, 2850, 1645, 1625, 1600, 1540, 975. ^{1}H-NMR (DMSO-d_6+$CDCl_3$) δ: 0.90 (br t, 3H, $J=6$ Hz), 2.10 (br, 2H), 2.65 (t, 2H, $J=7$ Hz), 3.10 (s, 2H), 3.40 (dt, 2H, $J=7$, 7 Hz), 5.85 (d, 1H, $J=16$ Hz), 5.65 (dt, 1H, $J=16$, 7 Hz), 7.40 (br, 1H), 7.90 (br, 2H).

Synthesis of Bis[(2-(*E*-2-nonenoylaminoethyl)] Disulfide (I-4)——The procedures used for I-1 were repeated with *trans*-2-nonenoic acid (5.0 g) to obtain I-4 (1.9 g, 27%). mp 127—129 °C (white crystals). IR ν_{max}^{KBr} cm^{-1}: 3300, 3150, 2930, 2850, 1665, 1620, 1540, 1465, 975. ^{1}H-NMR ($CDCl_3$) δ: 0.90 (br t, 6H, $J=6$ Hz), 2.20 (br, 4H), 2.90 (t, 4H, $J=6$ Hz), 3.65 (dt, 4H, $J=6$, 6 Hz), 5.90 (d, 2H, $J=15$ Hz), 6.90 (dt, 2H, $J=15$, 6 Hz), 6.7 (br, 2H).

Synthesis of 2-(*E*-2-Nonenoylamino)ethyl Carbamoylmethyl Sulfide (II-4)——The procedures used for II-1 were repeated with I-4 (1.0 g) to obtain II-4 (1.0 g, 78%). mp 150—151 °C (white crystals). IR ν_{max}^{KBr} cm^{-1}: 3370, 3300, 3190, 2930, 2840, 1665, 1640, 1620, 1545, 1490, 975. ^{1}H-NMR (DMSO-d_6+$CDCl_3$) δ: 0.88 (br, 3H), 2.15 (br, 2H), 2.70 (t, 2H, $J=7$ Hz), 3.12 (s, 2H), 3.20 (t, 2H, $J=7$ Hz), 5.85 (d, 1H, $J=16$ Hz), 6.68 (dt, 1H, $J=16$, 6 Hz), 6.9 (br, 1H), 7.35 (br, 1H), 7.88 (br, 1H).

Synthesis of Bis[(2-(*E*-2-decenoylaminoethyl)] Disulfide (I-5)——The procedures used for I-1 were repeated with *trans*-2-decenoic acid (15 g) to obtain I-5 (10.75 g, 75%). mp 136—136.5 °C (white crystals). IR ν_{max}^{KBr} cm^{-1}: 3300, 3050, 2920, 2850, 1665, 1620, 1540, 975. ^{1}H-NMR ($CDCl_3$+CF_3COOH) δ: 0.90 (br, 6H), 2.30 (br, 4H), 2.90 (t, 4H, $J=7$ Hz), 3.80 (t, 4H, $J=7$ Hz), 6.00 (d, 2H, $J=15$ Hz), 7.10 (dt, 2H, $J=15$, 16 Hz), 7.20 (br, 2H).

Synthesis of 2-(*E*-2-Decenoylamino)ethyl Carbamoylmethyl Sulfide (II-5)——The procedures used for II-1 were repeated with I-5 (2.0 g) to obtain II-5 (2.1 g, 84%). mp 151—151.5 °C (white crystals). IR ν_{max}^{KBr} cm^{-1}: 3370, 3300, 3170, 2920, 2850, 1650, 1620, 1540, 970. ^{1}H-NMR (DMSO-d_6+$CDCl_3$) δ: 0.90 (br t, 3H, $J=6$ Hz), 2.15 (br, 2H), 2.65 (t, 2H, $J=6$ Hz), 3.10 (s, 2H), 3.40 (dt, 2H, $J=6$, 6 Hz), 5.80 (d, 1H, $J=16$ Hz), 6.65 (dt, 1H, $J=16$, 7 Hz), 6.90 (br, 1H), 7.30 (br, 1H), 7.90 (br, 1H).

Synthesis of Bis[(2-(*E*-2-undecenoylaminoethyl)] Disulfide (I-6)——The procedures used for I-1 were repeated with *trnas*-2-undecenoic acid (12.3 g) to obtain I-6 (9.14 g, 68%). mp 133—133.5 °C (white crystals). IR ν_{max}^{KBr} cm^{-1}: 3300, 3050, 2920, 2850, 1650, 1620, 1600, 1545, 957. ^{1}H-NMR ($CDCl_3$+CF_3COOH) δ: 0.90 (br t, 6H), 2.40 (br, 4H), 2.90 (t, 4H, $J=7$ Hz), 3.80 (br t, 4H, $J=7$ Hz), 6.10 (d, 2H, $J=16$ Hz), 7.10 (dt, 2H, $J=16$, 7 Hz), 7.80 (br, 2H).

Synthesis of 2-(*E*-2-Undecenoylamino)ethyl Carbamoylmethyl Sulfide (II-6)——The procedures used for II-1 were repeated with I-6 (2.0 g) to obtain II-6 (2.0 g, 81%). mp 150.5—151.0 °C (white crystals). IR ν_{max}^{KBr} cm^{-1}: 3350, 3300, 3150, 2950, 2850, 1650, 1620, 1540, 1460, 970. ^{1}H-NMR (DMSO-d_6+$CDCl_3$) δ: 0.90 (br t, 3H, $J=6$ Hz), 2.10 (br, 2H), 2.70 (t, 2H, $J=7$ Hz), 3.30 (s, 2H), 3.35 (dt, 2H, $J=7$, 7 Hz), 5.85 (d, 1H, $J=16$ Hz), 6.70 (dt, 1H, $J=16$, 7 Hz), 7.20 (br, 1H), 7.90 (br, 2H).

Synthesis of Bis[(2-(*E*-2-dodecenoylaminoethyl)) Disulfide (I-7)——The procedures used for I-1 were repeated with *trans*-2-dodecenoic acid (15 g) to obtain I-7 (10.0 g, 83%). mp 136—137 °C (pale red crystals). IR ν_{max}^{KBr} cm^{-1}: 3300, 3050, 2930, 2850, 1665, 1620, 1545, 1465, 980. ^{1}H-NMR ($CDCl_3$+CF_3COOH) δ: 0.90 (br t, 6H, $J=6$ Hz), 2.30 (br, 4H), 2.90 (t, 4H, $J=6$ Hz), 3.82 (br t, 4H, $J=6$ Hz), 6.02 (d, 2H, $J=16$ Hz), 7.12 (dt, 2H, $J=16$, 16 Hz), 7.80 (br, 2H).

Synthesis of 2-(*E*-2-Dodecenoylamino)ethyl Carbamoylmethyl Sulfide (II-7)——The procedures used for II-1 were repeated with I-7 (2.0 g) to obtain II-7 (2.0 g, 82%). mp 143—144 °C (needle-shaped white crystals). IR ν_{max}^{KBr} cm^{-1}: 3380, 3300, 3180, 2850, 1650, 1620, 1545, 1460, 975. ^{1}H-NMR ($CDCl_3$+CF_3COOH) δ: 0.90 (br, 3H), 2.30 (br, 2H), 2.90 (t, 2H, $J=6$ Hz), 3.48 (s, 2H), 3.72 (t, 2H, $J=6$ Hz), 6.02 (d, 1H, $J=15$ Hz), 7.12 (dt, 1H, $J=15$, 6 Hz), 6.80—8.20 (br, m, 3H).

Synthesis of Bis[(2-(*E*-2-tridecenoylaminoethyl)] Disulfide (I-8)——The procedures used for I-1 were repeated with *trans*-2-tridecenoic acid (8.4 g) to obtain I-8 (3.28 g, 67%). mp 131—134 °C (pale yellow crystals). IR ν_{max}^{KBr} cm^{-1}: 3300, 3050, 2950, 2850, 1670, 1630, 1550, 980. ^{1}H-NMR ($CDCl_3$+CF_3COOH) δ: 0.90 (br, 6H), 2.30 (br, 4H), 2.90 (br, 4H), 3.90 (br, 4H), 6.10 (br d, 2H, $J=15$ Hz), 7.20 (br, 2H).

Synthesis of 2-(*E*-2-Tridecenoylamino)ethyl Carbamoylmethyl Sulfide (II-8)——The procedures used for II-1 were repeated with I-8 (3.7 g) to obtain II-8 (2.04 g, 45%). mp 151—152 °C (pale yellow crystals). IR ν_{max}^{KBr} cm^{-1}: 3400, 3330, 3200, 2950, 2850, 1650, 1630, 1550, 1460, 980. ^{1}H-NMR ($CDCl_3$+CF_3COOH) δ: 0.90 (br, 3H), 2.40 (br, 2H), 3.00 (br, 2H), 3.53 (s, 2H), 3.80 (br, 2H), 6.10 (br d, 1H, $J=15$ Hz), 7.00—8.90 (br, m, 3H).

Synthesis of Bis[(2-(*E*-2-tetradecenoylaminoethyl)] Disulfide (I-9)——The procedures used for Compound I-1 were repeated with *trans*-2-tetradecenoic acid (15 g) to obtain I-9 (4.2 g, 25%). mp 134—136.5 °C (white crystals). IR ν_{max}^{KBr} cm^{-1}: 3300, 2860, 1665, 1625, 1545, 1465, 985. ^{1}H-NMR ($CDCl_3+CF_3COOH$) δ: 0.90 (br t, 6H, J=6 Hz), 2.35 (br, 4H), 2.92 (t, 4H, J=6 Hz), 3.84 (br t, 4H, J=6 Hz), 6.04 (d, 2H, J=16 Hz), 7.13 (dt, 2H, J=16, 7 Hz), 6.80—8.20 (br, m, 2H).

Synthesis of 2-(*E*-2-Tetradecenoylamino)ethyl Carbamoylmethyl Sulfide (II-9)——The procedures used for II-1 were repeated with I-9 (1.5 g) to obtain II-9 (1.5 g, 82%). mp 154—155 °C (white crystals). IR ν_{max}^{KBr} cm^{-1}: 3380, 3300, 3180, 2950, 2850, 1665, 1650, 1622, 1545, 1460. ^{1}H-NMR ($CDCl_3+CF_3COOH$) δ: 0.90 (br t, 3H, J=6 Hz), 2.35 (br, 2H), 2.90 (t, 2H, J=7 Hz), 3.49 (s, 2H), 3.74 (t, 2H, J=7 Hz), 6.05 (d, 1H, J=16 Hz), 7.12 (dt, 1H, J=16, 7 Hz), 6.80—8.10 (br, m, 3H).

Assay of Gastric Juice Secretion-Inhibitory Activity in Rats——The assay was performed as follows. Male Wistar rats weighing 160—180 g, fasted for 48 h, were anesthetized with ethyl ether and the pylorus was ligated. Each sample was dissolved or suspended in 5% gum arabic in saline and administered intraperitoneally immediately after pylorus ligation. As a control, 5% gum arabic solution alone was administered. At 4 h after the pylorus ligation, the contents of the stomach were collected and the volume of gastric juice, total acid output and total peptic activity were determined by the methods described in our previous paper.[1]

Experimental Gastric Ulcers in Rats——i) Stress-Induced Gastric Ulcer: Male Wistar rats weighing 230—250 g, fasted for 48 h, were used as experimental animals. The rats were subjected to stress following the method of Takagi and Okabe,[5] in which animals were immobilized in a stress cage and immersed vertically in a water bath at 22±1 °C to the level of the xiphoid process for 7 h. Each sample was suspended in 5% gum arabic or 10% HCO-60 (Nikko Chemicals) in saline and administered intraperitoneally or perorally immediately before the stress treatment. The animals were sacrificed 7 h later. The stomach was then removed, inflated with 10 ml of saline and immersed in 1% formalin solution for 5 min. This formalin treatment was performed in all the following experiments. Subsequently, the stomach was incised along the greater curvature and examined for gastric lesions.

The total length (mm) of all lesions in the glandular portion of the stomach was used as an ulcer index.

ii) Aspirin-Induced Gastric Ulcer: Male Wistar rats weighing 160—180 g were deprived of food for 24 h. According to the method of Okabe *et al.*,[6] the rats were orally given 150 mg/kg of aspirin (Nakarai Chemicals, Ltd.) suspended in 5% gum arabic solution immediately after the pylorus ligation. Each sample was suspended in 5% gum arabic in saline and administered intraperitoneally immediately after the pylorus ligation. At 7 h after the pylorus ligation, rats were sacrificed and their stomachs removed. After the formalin treatment, the lesions in the glandular portion of the stomach were examined and the sum of the lengths (mm) of all lesions was used as an ulcer index.

iii) HCl–Ethanol-Induced Gastric Ulcer: Male Wistar rats weighing 160—190 g, fasted for 24 h, were used as experimental animals. This ulcer model was induced according to the method of Mizui and Doteuchi.[7] Each rat received 1.5 ml of 150 mM HCl-60% ethanol solution. Test samples were suspended in 5% gum arabic in saline and administered intraperitoneally 2 h before the HCl–ethanol administration. The animals were sacrificed 1 h later and their stomachs were removed to examine the lesions in the glandular portion. The sum of the length (mm) of all lesions was used as an ulcer index.

Acute Toxicity Test in Mice——Acute toxicity was studied in male ddY mice, weighing 18—21 g. Five mice in each group were used for the experiment. Test samples, suspended in 10% HCO-60 in saline, were administered at doses of 125, 500, 2000 and 8000 mg/kg perorally or intraperitoneally at a volume of 40 ml/kg. After the administration of a single dose of each test sample, the behavior of the animals was also observed for 6 h, then they were caged and bred as usual for 7 d. Numbers of dead animals and the change of body weight in each mouse were observed every day until the end of the experiment.

Statistics——Results are expressed as the mean±S.E. Student's t test was applied to evaluate the significance of differences between the mean of the control group and the means of sample-administered groups.

Results

Inhibitory Effects of Various Sulfur-Containing Amino Acids and Amines on Gastric Secretion in Rats

The inhibitory effect of various samples, given at doses of 5, 10, and 25 mg/kg i.p., on gastric juice secretion in pylorus-ligated rats, was examined by measuring the volume, total acid output and total peptic activity of gastric juice, collected for 4 h during the experiment.

At a dose of 25 mg/kg i.p., all of the test sample-injected groups, except for the cysteine and cysteamine groups, showed a significant inhibition in the volume, total acid output and total peptic activity of gastric juice when compared to the control group. At a dose of 10 mg/kg i.p., only cystamine and lanthionine showed a significant gastric juice secretion-

TABLE I. Effect of Various Sulfur-Containing Amino Acids and Amines on Gastric Secretion in Pylorus-Ligated Rats (4 h)

Treatment	Dose (mg/kg)	Gastric volume (ml/100 g b.w.)	Total acid output (μeq/100 g b.w.)	Total peptic activity (mg as tyrosine/100 g b.w.)
1) Control[a]	—	2.89±0.22	201.8±11.6	206.8±10.3
Cystine	25	2.05±0.09[c]	146.4± 8.5[c]	165.5± 3.5[c]
Cysteine	25	2.53±0.13	171.0± 9.7	176.5±12.0
Methionine	25	2.13±0.20[b]	145.7± 6.9[c]	166.9± 8.8[b]
Taurine	25	1.72±0.08[d]	123.6± 4.2[d]	145.0± 3.5[d]
2) Control[a]	—	2.62±0.28	260.8±30.3	184.2±15.4
Hypotaurine	25	1.66±0.31[b]	157.3±39.3	114.5±15.9[c]
Ethionine	25	1.52±0.22[b]	105.9±18.6[d]	102.7±18.4[c]
Methionine sulfate	25	1.66±0.32[b]	136.5±33.7[b]	107.5±14.5[c]
3) Control[a]	—	2.79±0.35	271.1±39.0	232.8±26.7
Cystamine	25	1.36±0.16[c]	120.6±19.9[c]	125.6±14.2[c]
Cysteamine	25	2.02±0.26	165.8±31.0	175.0±22.2
Lanthionine	25	1.76±0.23	142.3±21.5[b]	152.9±16.4[b]
Methionine sulfone	25	1.72±0.30[b]	155.0±33.5	143.6±23.0[b]
4) Control[a]	—	2.26±0.24	223.5±29.0	194.2±20.2
Methionine	10	1.90±0.18	170.7±19.2	148.4±22.4
Ethionine	10	1.89±0.26	191.8±23.7	228.0±24.7
Lanthionine	10	1.02±0.20[c]	122.9±16.6[c]	152.1±18.6
Methionine sulfone	10	1.64±0.18	186.9±19.8	182.8±18.9
Cystamine	10	0.78±0.14[d]	84.0±14.5[d]	110.3±10.9[c]
5) Control[a]	—	2.90±0.26	371.3±35.1	235.2±20.2
Cystine	10	2.54±0.30	328.2±33.4	190.2±22.9
Taurine	10	3.04±0.20	379.0±38.6	224.6±12.3
Hypotaurine	10	3.14±0.26	413.5±41.3	237.4±19.1
Methionine sulfate	10	2.49±0.39	310.2±56.3	184.5±13.9
6) Control[a]	—	2.60±0.23	288.8±38.7	205.3±17.1
Lanthionine	5	2.92±0.25	339.3±25.1	204.4±14.7
Cystamine	5	2.51±0.14	286.2±18.1	190.8±10.0

All values represent the mean±S.E. of 8 rats. *a*) Acacia, 5% in saline. Each sample was intraperitoneally administered immediately after pylorus ligation. Significantly different from the control group: *b*) $p<0.05$, *c*) $p<0.01$, *d*) $p<0.001$.

inhibitory activity; they gave 62% and 45% inhibitions of total acid output, respectively. Cystamine was more potent than lanthionine, but the difference was not significant.

Thus, cystamine was the strongest gastric secretion-inhibitory material among the various sulfur-containing amino acids and amines tested (Table I).

Inhibitory Effects of Several Disulfides (I-1—I-9) and Sulfides (II-1—II-9) on Gastric Secretion in Rats

In order to examine the gastric juice secretion-inhibitory activity for each of the eighteen synthesized derivatives, each test sample was given intraperitoneally to rats at a dose of *25 mg/kg immediately after pylorus ligation.*

All sulfides (II), which were alkylated with iodoacetamide, showed stronger activity than the disulfides (I), no matter what the length of the alkenyl chains (Table II). The correlation between the length of the alkenyl chain of the unsaturated fatty acid in II-1—II-9 and the total acid output inhibitory activity was examined; the results are shown in Fig. 1. Compounds II-5 and II-6, containing 10 or 11 carbons in the alkenyl chain of the unsaturated fatty acid, showed stronger inhibitory activity than cystamine or unsaturated fatty acid having the same carbon number alone.

We found a correlation between the pharmacological activity and the carbon number of

TABLE II. Effect of Various Bis[2-(*E*-2-alkenoylaminoethyl)] Disulfides and 2-(*E*-2-Alkenoylamino)ethyl Carbamoylmethyl Sulfides on Gastric Secretion in Pylorus-Ligated Rats (4 h)

Treatment	Dose (mg/kg)	Gastric volume (ml/100 g b.w.)	Total acid output (μeq/100 g b.w.)	Total peptic activity (mg as tyrosine/100 g b.w.)
Control[a)]	—	3.05±0.20	326.0±26.1	184.5±13.7
I-1	25	2.61±0.28	287.7±33.7	161.7±16.9
II-1	25	2.32±0.38	253.3±53.1	157.4±21.7
I-2	25	2.50±0.35	267.9±48.5	136.0±13.3
II-2	25	2.14±0.41	194.7±38.0[b)]	138.2±19.5
Control[a)]	—	3.50±0.24	394.5±36.5	214.0±15.4
I-3	25	2.72±0.29	289.8±36.2	156.8±11.9
II-3	25	1.95±0.41[c)]	180.2±39.8[d)]	120.1±18.6[c)]
I-4	25	3.09±0.29	318.6±33.8	177.9±15.3
II-4	25	1.75±0.44[c)]	170.1±49.0[c)]	120.4±24.8[c)]
Cystamine	25	1.80±0.39[d)]	192.2±49.6[c)]	129.9±20.8[c)]
Control[a)]	—	2.49±0.32	289.3±36.5	150.9±13.5
I-5	25	2.44±0.36	320.8±54.9	150.2±19.4
II-5	25	0.93±0.13[d)]	91.1±15.1[d)]	81.5± 8.1[d)]
I-9	25	1.68±0.38	187.5±49.6	125.4±23.0
II-9	25	1.90±0.30	207.6±35.9	135.1±16.6
Control[a)]	—	2.98±0.26	331.4±34.9	184.2±13.2
I-6	25	2.10±0.26[b)]	229.2±30.5[b)]	139.9±15.8[b)]
II-6	25	0.97±0.20[d)]	97.4±24.3[d)]	70.9±11.1[d)]
I-7	25	2.20±0.43	243.5±49.7	143.3±22.7
II-7	25	1.50±0.24[d)]	167.9±31.9[c)]	108.3±12.3[d)]
Cystamine	25	1.42±0.16[d)]	150.0±18.9[d)]	99.0± 7.8[d)]
Control[a)]	—	2.37±0.36	223.4±58.0	138.4±15.3
I-8	25	1.66±0.41	177.9±50.3	116.4±21.0
II-8	25	1.02±0.14[c)]	108.1±18.1	73.9± 7.4[c)]

All values represent the mean±S.E. of 8 rats. *a*) Acacia, 5% in saline. Each sample was intraperitoneally administered immediately after pylorus ligation. Significantly different from the control group: *b*) $p<0.05$, *c*) $p<0.01$, *d*) $p<0.001$.

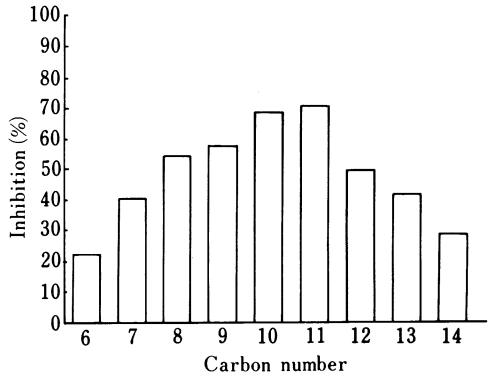


Fig. 1. Effect of Carbon Number of Unsaturated Fatty Acids in Various 2-(*E*-2-Alkenoylamino)ethyl Carbamoylmethyl Sulfides on Gastric Secretion in Pylorus-Ligated Rats (4 h)

Each column represents percent inhibition of total acid output in various 2-(*E*-2-alkenoylamino)ethyl carbamoylmethyl sulfide-treated groups against that in the control group. Each sample was administered intraperitoneally (25 mg/kg) immediately after pylorus ligation.

the unsaturated fatty acid in the synthesized sulfides. Namely, the peak of gastric secretion-inhibitory activity was observed at 10 or 11 carbons in the unsaturated fatty acid (Fig. 1).

Anti-ulcer Activity of 2-(*E*-2-Decenoylamino)ethyl Carbamoylmethyl Sulfide (II-5) and 2-(*E*-2-Undecenoylamino)ethyl Carbamoylmethyl Sulfide (II-6) on Various Experimental Ulceration Models in Rats

The effects of II-5 and II-6, which exhibited the strongest gastric secretion-inhibitory

TABLE III. Effect of 2-(*E*-2-Decenoylamino)ethyl Carbamoylmethyl Sulfide and 2-(*E*-2-Undecenoylamino)ethyl Carbamoylmethyl Sulfide on Stress-, Aspirin- and HCl·Ethanol-Induced Ulceration in Rats

Ulceration	Treatment	Dose (mg/kg)	Ulcer index	Inhibition (%)
Stress-induced ulceration (7 h)	Control[a]	—	29.1 ± 3.0	—
	II-5[b]	20	13.3 ± 5.1[e]	54.3
	II-6[b]	20	9.0 ± 2.7[f]	69.1
	Cimetidine[b]	80	5.4 ± 1.8[g]	81.4
Aspirin-induced ulceration (7 h)	Control[a]	—	17.1 ± 2.8	—
	II-5[c]	20	8.6 ± 2.5[e]	49.7
	II-6[c]	20	4.0 ± 1.1[g]	76.4
	Cimetidine[c]	80	2.9 ± 0.7[g]	83.0
HCl·ethanol-induced ulceration (1 h)	Control[a]	—	41.3 ± 7.4	—
	II-5[d]	20	12.5 ± 3.0[f]	69.7
	II-6[d]	20	12.2 ± 3.4[f]	70.5
	Cimetidine[d]	80	31.3 ± 12.7	24.2

All values represent the mean ± S.E. of 8 rats. *a*) Acacia, 5% in saline. *b*) Each sample was intraperitoneally administered immediately before the restraint and water-immersion stress loading. *c*) Each sample was intraperitoneally administered immediately after pylorus ligation. *d*) Each sample was intraperitoneally administered 2 h before the per oral administration of 150 mM HCl, 60% ethanol (1.5 ml/rat) to rats. Significantly different from the control group: *e*) $p<0.05$, *f*) $p<0.01$, *g*) $p<0.001$.

activity, on water-immersion stress-, aspirin-, and HCl–ethanol-induced gastric ulcers in rats were examined.

Stress-Induced Gastric Ulcer——At 20 mg/kg i.p., the ulcer index of the animals treated with II-5 and II-6 was significantly less ($p<0.05$) than that of the control group, and the inhibitory activity of II-6 was more potent than that of II-5. Cimetidine was used as a positive control (Table III).

Aspirin-Induced Gastric Ulcer——As shown in Table III, II-5 and II-6 showed a significant decrease in their ulcer indices at a dose of 20 mg/kg i.p., and II-6 was more effective than II-5. Compound II-6 showed almost the same activity as cimetidine, which was used as a positive control.

HCl–Ethanol-Induced Gastric Ulcer——Both II-5 and II-6 showed the same inhibitory ratio (about 70%) for HCl–ethanol-induced gastric ulcer at a dose of 20 mg/kg i.p. Cimetidine, used as a positive control, did not show a significant inhibition, even at a dose of 80 mg/kg i.p.

Effect of Dispersive Agents for II-5 and II-6 on Stress-Induced Gastric Ulcer in Rats

Compounds II-5 and II-6 were hardly soluble in water, so we used them as a suspension in 5% gum arabic solution. In order to examine the effect of dispersive agents for these two derivatives, we used gum arabic and HCO-60 (a detergent; Nikko Chemicals), and compared their inhibitory effects on water immersion stress-loaded rats. Peroral administration, in 5% gum arabic, gave a weaker activity than intraperitoneal administration; however, the inhibition of ulceration was substantial (about 50—60%) at a dose of 20 mg/kg. At a dose of 5 mg/kg, the inhibition was as low as 40% (not significantly different from the control).

Compounds II-5 and II-6 showed more potent inhibitory activity when they were suspended in 10% HCO-60 in saline than when they were suspended in 5% gum arabic solution (Table IV).

The relationship between the administered dose and the antiulcer activity of II-5 and II-6 was examined by using restrained and water-immersion stress-loaded rats. Both acetamides

TABLE IV. Effect of 2-(*E*-2-Decenoylamino)ethyl Carbamoylmethyl Sulfide and 2-(*E*-2-Undecenoylamino)ethyl Carbamoylmethyl Sulfide on Stress-Induced Ulceration in Rats

Treatment	Dose (mg/kg)	Ulcer index	Inhibition (%)
Control[a]	—	16.8±2.6	—
II-5	20	8.4±1.1[c]	50.0
	5	9.7±2.1	42.3
II-6	20	6.7±0.8[d]	60.1
	5	10.4±1.6	38.1
Control[b]	—	18.1±3.7	—
II-5	20	7.9±1.2[c]	56.4
	5	9.2±2.5	49.2
II-6	20	6.7±1.1[d]	63.0
	5	8.4±1.9[c]	53.6

All values represent the mean±S.E. of 8 rats. *a*) Acacia, 5% in saline. *b*) HCO-60, 10% in saline. Each sample was perorally administered immediately before the restraint and water-immersion stress loading. Significantly different from the control group: *c*) $p<0.05$, *d*) $p<0.01$.

TABLE V. Effect of Various 2-(*E*-2-Alkenoylamino)ethyl Carbamoylmethyl Sulfides on Stress-Induced Ulceration in Rats

Treatment	Dose (mg/kg)	Ulcer index	Inhibition (%)
Control[a]	—	15.1±1.3	—
II-1	5	13.3±2.3	11.9
II-5	5	7.5±1.4[c]	50.3
II-6	5	7.0±1.1[d]	53.6
Control[a]	—	15.6±1.9	—
II-2	5	10.8±1.7	30.9
II-3	5	11.4±1.2	26.6
II-4	5	9.4±1.5[b]	39.5
Control[a]	—	18.6±2.4	—
II-7	5	14.3±2.8	23.1
II-8	5	17.6±1.4	5.4
II-9	5	18.5±3.2	0.5

All values represent the mean±S.E. of 8 rats. *a*) HCO-60, 10% in saline. Each sample was perorally administered immediately before the restraint and water-immersion stress loading. Significantly different from the control group: *b*) $p<0.05$, *c*) $p<0.01$, *d*) $p<0.001$.

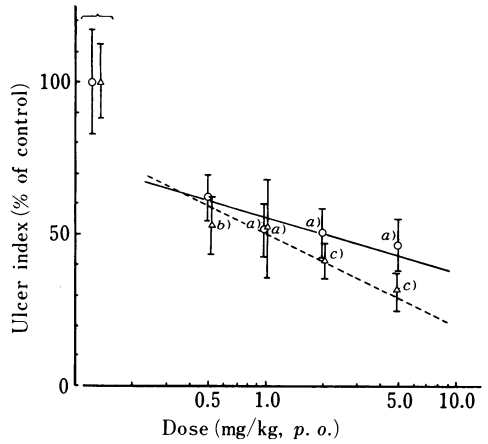


Fig. 2. Dose-Response Curves for the Inhibitory Effect of 2-(*E*-2-Decenoylamino)ethyl Carbamoylmethyl Sulfide and 2-(*E*-2-Undecenoylamino)ethyl Carbamoylmethyl Sulfide on Stress-Induced Ulceration in Rats

Each point represents the mean±S.E. of 8 rats. Significantly different from each control group: *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$. ○—○, II-5; △---△, II-6; ⌒⌒, control (10% HCO-60 in saline).

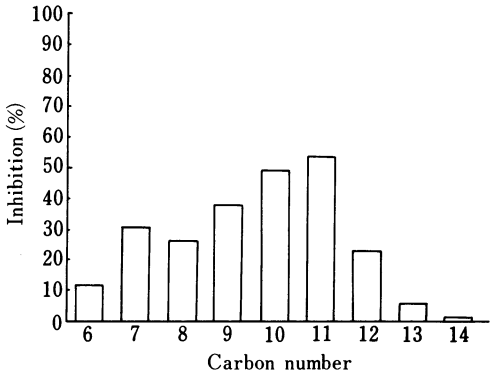


Fig. 3. Effect of Carbon Number of Unsaturated Fatty Acids in Various 2-(*E*-2-Alkenoylamino)ethyl Carbamoylmethyl Sulfides on Stress-Induced Ulceration in Rats (7 h)

Each column represents percent inhibition of ulcer index in various 2-(*E*-2-alkenoylamino)ethyl carbamoylmethyl sulfide-treated groups against that in the control group. Each sample was administered perorally (5 mg/kg) immediately before the restraint and water-immersion stress loading.

showed a dose-dependent decrease of the ulcer index between the dosage range from 0.5 to 5 mg/kg *p.o.* (Fig. 2). The ED_{50} values (Fig. 2) were about 2 mg/kg (1—5 mg/kg) for II-5 and about 1 mg/kg (0.5—2.5 mg/kg) for II-6.

Effect of Carbon Number of Unsaturated Fatty Acid in Various Sulfides on Stress-Induced Gastric Ulceration in Rats

Compounds II-1—II-9 were suspended with 10% HCO-60 solution and administered at a

dose of 5 mg/kg *p.o.* to rats, which were then restrained for 7 h during water-immersion stress loading. The ulcer indices obtained are shown in Table V. The correlation between the inhibition ratio (%) of ulcer index (treated *vs.* control) and the alkenyl chain length of the unsaturated fatty acid in various sulfides is summarized in Fig. 3.

The strongest inhibitory activity was observed between the carbon numbers of 10 and 11 gave the most potent activity.

Acute Toxicity Test of II-5 and II-6 in Mice

No deaths or toxic symptoms were observed in mice at any dose examined (*p.o.* or i.p.) during the experimental period. The increase of body weight was unaffected when the test samples were administered perorally. However, when the test samples were administered intraperitoneally, a slight inhibition of body weight gain was observed a few days after the sample administration in the higher dosage groups (8000 and 2000 mg/kg) of both compounds.

Discussion

We have found that reduction and alkylation of interchain disulfide bonds of native IgG result in the appearance of gastric secretion-inhibitory activity. Alkylation of thiols was necessary, because the heavy and light chains in which SH groups were left free showed no inhibitory activity.[1)]

In the research described in this paper, we studied the effect of modification of the SH group of simple thiol-containing amino acids and amines. Comparison of cystine and cysteine, cystamine and cysteamine indicated that the compounds which had free SH groups showed no anti-gastric secretion activity; compounds which had masked SH groups were, however, effective. Among various amino acids and amines containing a thiol, cystamine showed the strongest activity. We have already reported that *trans*-2-unsaturated fatty acids inhibited gastric secretion.[4)] Therefore, we prepared derivatives of cystamine using *trans*-2-unsaturated fatty acids with various chain lengths. Disulfide (I) and sulfide (II) derivatives were screened for pharmacological activity, and it was found that compounds (II) which has a carbamoylmethylated sulfide moiety showed stronger inhibitory activity than those having disulfides (I) in the stress ulceration model. Moreover, there was a relationship between the chain length of 2-alkenoic acid and the biological activity; the compounds containing a 2-alkenoic acid with a chain length of 10 to 11 carbon atoms (II-5, II-6) showed the strongest activity.

Therefore, in the next step, we tested II-5 and II-6 in other experimental ulcer models induced by aspirin and HCl–ethanol. Both compounds showed potent inhibitory activity in both models. In the formation of stress-induced, pylorus-ligation and aspirin-induced ulcers, the reduction of gastric membrane defensive factors is reported to be important, in addition to the influence of gastric acid as an offensive factor.[8,9)] Further study will be necessary to elucidate the mechanism of action of our compounds.

These compounds also showed anti-ulcerogenic activity when the administration route was changed from peritoneal to peroral, at a dose of 20 mg/kg in the water-immersed stress model. At a dose of 5 mg/kg, both compounds showed approximately 40% inhibition, which was not statistically significant when compared with the control group. However, when dispersed in 10% HCO-60 and administered orally, both compounds showed more potent activity than when administered with 5% gum arabic. The results suggested that the compounds were better dispersed in 10% HCO-60 than 5% gum arabic and were better absorbed.

A dose-dependent decrease of ulcer index was observed in the restrained and water-immersion stress-loaded rats at the dosage range from 0.5 to 5 mg/kg *p.o.* for both

compounds. This activity is comparable with that of commonly used anti-peptic ulceration drugs.

Since no toxic symptoms were observed at any dose tested in mice (perorally or intraperitoneally), these derivatives may be practically useful as anti-peptic-ulceration drugs.

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