Inhibitory Effect of 2-(*E*-2-Alkenoylamino)ethyl Alkyl Sulfides on Gastric Ulceration in Rats. II. Structure and Activity Relationships of 2-(*E*-*n* or *Z*-*n*-Decenoylamino)ethyl Alkyl Sulfides

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The analogues of 2-(*E*-*n* or *Z*-*n*-decenoylamino)ethyl carbamoylmethyl sulfide, including the modifications of sulfide portion, double bond in decenoyl chain and alkyl sulfide moiety, were synthesized and their inhibitory effects on stress-induced ulceration in rats were compared.

Replacing the sulfura atom by methylene group or oxygen atom reduced the effect of potency. Saturation of the double bond in the decenoyl chain tended to reduce the anti-ulcerogenic activity in rats. There was no relationship between the position of double bond in decenoyl chain and the pharmacological activity. On the other hand, compounds with *E*-configuration showed stronger anti-ulcer activity than the corresponding *Z*-type of compounds. Among 9 kinds of *S* substituted alkyl groups for carbamoylmethyl, 2-(*E*-2-decenoylamino)ethyl 2-cyclohexylethyl sulfide showed the most potent anti-ulcerogenic activity in rats and also showed the lowest acute toxicity in mice.

Keywords 2-(*E*-2-alkenoylamino)ethyl alkyl sulfide; 2-(*E*-2-decenoylamino)ethyl cyclohexylethyl sulfide; anti-ulcerogenic activity; acute toxicity

We have reported that human immunoglobulin G (IgG) showed anti-ulcerogenic¹⁾ and anti-inflammatory²⁾ activities after reductive cleavage of interchain disulfide bonds, though the native IgG showed no such activities. It was suggested that chemical modification of S–S bonds in the hinge region was essential to the appearance of these pharmacological activities. We have also reported the anti-ulcerogenic activities of various saturated³⁾ and unsaturated⁴⁾ fatty acids and investigated the relationship between alkyl or alkenyl chain lengths and pharmacological activities. In an effort to develop a new anti-ulcerogenic drug based on this evidence, we previously investigated the pharmacological activities of a series of 2-(*E*-2-alkenoylamino)ethyl alkyl sulfides which were synthesized from various *E*-2-unsaturated fatty acids and cystamine.⁵⁾ In this series, 2-(*E*-2-decenoylamino)ethyl carbamoylmethyl sulfide (I-1-a) was the most potent inhibitor of gastric ulceration in rats.

In the present article, a structure–activity study of I-1-a has been made in an attempt to test the effects of modifications of the sulfide portion, double bond in decenoyl chain and alkyl sulfide moiety on the appearance of the anti-ulcerogenic activity.

Experimental

Synthesis of 2-(*E*-2-Decenoylamino)ethyl Carbamoylmethyl Sulfide (I-1-a) Neat SOCl₂ was added dropwise to a *E*-2-decenoic acid at 0°C. This solution was stirred for 4 h under reflux, and then the excess SOCl₂ was removed by evaporation. The residual oil was purified by distillation, affording *E*-2-decenoyl chloride as a pale yellow oil. One normal NaOH was added to a stirred suspension of cystamine dihydrochloride in water at 0°C to pH 8.0. The solution was added with *E*-2-decenoyl chloride in CH₂Cl₂, and stirred for 1 h at room temperature. After the reaction, ethyl acetate (EtOAc) and 0.5 N HCl were added to the solution and suspended. The organic layer was removed and washed with water, 5% NaHCO₃, water, and brine. The EtOAc solution was dried over MgSO₄ and evaporated. The residual solid was crystallized from EtOAc, affording the *N,N'*-bis(*E*-2-decenoyl)cystamine. Bu₃P was added to a stirred suspension of this compound in 90% MeOH/water at 0°C, and stirred for 1 h at room temperature. Iodoacetamide and 1 N NaOH were added to the solution and stirred for 1.5 h at room temperature. After the reaction, an off-white solid was precipitated by the addition of water. The suspension was filtered, and the solid was washed with water, affording I-1-a as a white crystal,

mp 151.0–151.5°C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3370, 3300, 3170, 2920, 2850, 1650, 1620, 1540, 970. ¹H-NMR (DMSO-*d*₆ + CDCl₃) δ : 0.90 (3H, br t, *J* = 6 Hz), 2.15 (2H, br s), 2.65 (2H, t, *J* = 6 Hz), 3.10 (2H, s), 3.40 (2H, br dt, *J* = 6.6 Hz), 5.80 (1H, d, *J* = 16 Hz), 6.65 (1H, dt, *J* = 16, 7 Hz), 6.90 (br t, 1H), 7.30 (br s, 1H), 7.90 (br s, 1H). HRMS *m/z*: 287.1745 [M + H] (287.1792 Calcd for C₁₄H₂₆N₂O₂S + H).

Synthesis of 5-(*E*-2-Decenoylamino)pentanamide (I-2-a) Neat SOCl₂ was added dropwise to 5-bromopentanoic acid at 0°C. This solution was stirred for 4 h under reflux, and then the excess SOCl₂ was removed by evaporation. The residual oil was purified by distillation, affording 5-bromopentanoyl chloride as an oil. IR $\nu_{\text{max}}^{\text{film}}$ cm⁻¹: 1800. 5-Bromopentanoyl chloride was added to a stirred solution of 28% NH₄OH at 0°C, and stirred for 45 min at the same temperature. Chloroform was added and suspended. The organic layer was removed, and the aqueous layer was extracted with chloroform. The chloroform layer was combined, washed with brine, dried over MgSO₄ and then evaporated, affording 5-bromopentanamide as a colorless crystal. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1650, 1630. Dibenzylamine and anhydrous K₂CO₃ were added to a stirred solution of 5-bromopentanamide in dry dimethylformamide (DMF), and stirred for 87 h at room temperature. EtOAc and water was added to the solution and suspended. The organic layer was removed, and the aqueous layer was extracted with EtOAc. The EtOAc layer was combined, washed with water and brine, dried over MgSO₄, and then evaporated. The residue was chromatographed on silica gel (9:1 EtOAc–MeOH), affording 5-dibenzylaminopentanamide. A solution of 5-dibenzylaminopentanamide in MeOH was treated with 10% Pd/C and stirred under H₂ atmosphere for 43 h. The suspension was filtered through celite and evaporated, affording 5-aminopentanamide. 2-Decenoyl chloride was added dropwise to a stirred solution of 5-aminopentanamide in 0.6 N NaOH at 0°C, and stirred for 30 min at room temperature. The suspension was filtered, and the solid was washed with water and ether. The solid was dried *in vacuo*, affording I-2-a as a needle-shaped white crystal, mp 184.0–185.5°C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3360, 3400, 3180, 1645, 1630. ¹H-NMR (DMSO-*d*₆) δ : 0.86 (3H, m), 1.10–1.60 (14H, m), 2.00–2.20 (4H, m), 3.00–3.13 (2H, m), 5.86 (1H, d, *J* = 15.3 Hz), 6.58 (1H, dt, *J* = 15.3, 6.8 Hz), 6.63–6.80 and 7.18–7.30 (2H, br s), 7.80–7.90 (1H, br t). HRMS *m/z*: 269.2231 [M + H] (269.2227 Calcd for C₁₅H₂₈N₂O₂ + H).

Synthesis of 2-(*E*-2-Decenoylamino)ethyl Carbamoylmethyl Ether (I-3-a) 1.58 N *n*-BuLi/Hex was added dropwise to a stirred solution of dibenzylamine in dry hexamethylphosphoramide (HMPA) and dry tetrahydrofuran (THF) at –70°C. The scarlet solution was then stirred for 45 min at the same temperature. Ethylenedibromohydrin in dry THF was added dropwise to the solution. After the addition, the solution was gradually warmed to room temperature, dry EtOAc was added, and the solution was stirred for 30 min. The solution was diluted with EtOAc, washed with water and brine, dried over MgSO₄, and then evaporated. The residual oil was chromatographed on silica gel (1:3 EtOAc–hexane), affording *O*-acetyl-*N,N*-dibenzylethanolamine as a colorless oil. IR $\nu_{\text{max}}^{\text{film}}$

cm^{-1} : 1740. Anhydrous K_2CO_3 was added to a stirred solution of *O*-acetyl-*N,N*-dibenzylethanamine in MeOH and stirred for 30 min at room temperature. EtOAc and water added to the solution and suspended. The organic layer was removed, washed with water and brine, dried over MgSO_4 , and then evaporated. The residual oil was chromatographed on silica gel (1:2 EtOAc-hexane), affording *N,N*-dibenzylethanamine. IR $\nu_{\text{max}}^{\text{film}}$ cm^{-1} : 3400. A suspension of 35% KH in oil was washed with hexane and dry THF was added to it. *N,N*-Dibenzylethanamine in dry THF was added dropwise at 0°C to the suspension. After the addition, iodoacetamide in dry THF was added dropwise to the solution, and stirred overnight. EtOAc, water and 1N NaOH were added and suspended. The organic layer was removed, washed with water and brine, dried over MgSO_4 , and then evaporated. The residue was chromatographed on silica gel (9:1 EtOAc-MeOH), affording *N,N*-dibenzyl-*O*-carbamoylmethyl-ethanolamine as a crystal. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3450, 3200, 1680. A solution of *N,N*-dibenzyl-*O*-carbamoylmethyl-ethanolamine in MeOH was treated with 10% Pd/C and stirred under H_2 atmosphere for 19 h. The suspension was filtered through celite and evaporated, affording *O*-carbamoylmethyl-ethanolamine. *E*-2-Decenoyl chloride was added dropwise to a stirred solution of *O*-carbamoylmethyl-ethanolamine in 1N NaOH and water at 0°C , and stirred for 30 min at room temperature. EtOAc was added to the solution and suspended. The organic layer was removed, and the aqueous layer was extracted with EtOAc. The EtOAc layer was combined, washed with water and brine, dried over MgSO_4 , and then evaporated. The residual solid was chromatographed on octadecyl silica (ODS) [high performance liquid chromatography (HPLC)], affording I-3-a as a crystal powder, mp $129.0\text{--}130.0^\circ\text{C}$. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3410, 3310, 3200, 1630, 1225. $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : 0.86 (3H, m), 1.10–1.50 (10H, m), 2.05–2.20 (2H, m), 3.25–3.38 (2H, m), 3.46 (2H, t, $J=5.2\text{ Hz}$), 3.80 (2H, s), 5.90 (1H, d, $J=15.4\text{ Hz}$), 6.62 (1H, dt, $J=15.4, 6.8\text{ Hz}$), 7.10–7.45 (2H, brs), 8.01 (1H, brt). HRMS m/z : 271.1975 [M+H] (271.2020 Calcd for $\text{C}_{14}\text{H}_{26}\text{N}_2\text{O}_3\text{S}+\text{H}$).

Synthesis of 2-Decanoylaminoethyl Carbamoylmethyl Sulfide (I-1-b)
The procedures used for I-1-a were repeated with decanoic acid, affording I-1-b as a white crystal, mp $141.0\text{--}142.0^\circ\text{C}$. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3370, 3320, 3170, 1650, 1540. $^1\text{H-NMR}$ ($\text{CF}_3\text{COOH}+\text{CDCl}_3$) δ : 0.89 (3H, brt, $J=6\text{ Hz}$), 1.10–2.00 (14H, m), 2.30–3.20 (4H, m), 3.48 (2H, s), 3.60–3.90 (2H, m), 7.80 (1H, brt), 8.10 (2H, brs). HRMS m/z : 289.1920 [M+H] (289.1948 Calcd for $\text{C}_{14}\text{H}_{26}\text{N}_2\text{O}_2\text{S}+\text{H}$).

Synthesis of 2-(E-3-Decenoylamino)ethyl Carbamoylmethyl Sulfide (I-1-c)
The procedures used for I-1-a were repeated with *E*-3-decenoic acid, affording I-1-c as a crystal powder, mp $126.5\text{--}127.0^\circ\text{C}$. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3380, 3320, 3180, 1640, 1540, 960. $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : 1.90–2.03 (2H, m), 2.56–2.66 (2H, m), 2.79 (2H, d, $J=4.4\text{ Hz}$), 3.08 (2H, s), 3.16–3.27 (2H, m), 5.35–5.60 (2H, m). HRMS m/z : 287.1794 [M+H] (287.1792 Calcd for $\text{C}_{14}\text{H}_{26}\text{N}_2\text{O}_2\text{S}+\text{H}$).

Synthesis of 2-(Z-3-Decenoylamino)ethyl Carbamoylmethyl Sulfide (I-1-d)
The procedures used for I-1-a were repeated with *Z*-3-decenoic acid, affording I-1-d as a crystal powder, mp $116.0\text{--}117.0^\circ\text{C}$. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3380, 3320, 3180, 1640, 1540. $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : 1.95–2.07 (2H, m), 2.56–2.65 (2H, m), 2.87 (2H, d, $J=5.8\text{ Hz}$), 3.08 (2H, s), 3.17–3.28 (2H, m), 5.36–5.56 (2H, m). HRMS m/z : 287.1812 [M+H] (287.1792 Calcd for $\text{C}_{14}\text{H}_{26}\text{N}_2\text{O}_2\text{S}+\text{H}$).

Synthesis of 2-(E-4-Decenoylamino)ethyl Carbamoylmethyl Sulfide (I-1-e)
The procedures used for I-1-a were repeated with *E*-4-decenoic acid, affording I-1-e as a crystal powder, mp $140.0\text{--}142.0^\circ\text{C}$. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3370, 3300, 1645, 1635, 960. $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : 1.87–2.00 (2H, m), 2.00–2.24 (4H, m), 2.56–2.65 (2H, m), 3.08 (2H, s), 3.17–3.28 (2H, m), 5.28–5.50 (2H, m). HRMS m/z : 287.1807 [M+H] (287.1792 Calcd for $\text{C}_{14}\text{H}_{26}\text{N}_2\text{O}_2\text{S}+\text{H}$).

Synthesis of 2-(Z-4-Decenoylamino)ethyl Carbamoylmethyl Sulfide (I-1-f)
The procedures used for I-1-a were repeated with *Z*-4-decenoic acid, affording I-1-f as crystal powder, mp $121.5\text{--}124.0^\circ\text{C}$. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3370, 3310, 1625, 1615. $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : 1.90–2.27 (6H, m), 2.55–2.64 (2H, m), 3.07 (2H, s), 3.16–3.27 (2H, m), 5.21–5.41 (2H, m). HRMS m/z : 287.1828 [M+H] (287.1792 Calcd for $\text{C}_{14}\text{H}_{26}\text{N}_2\text{O}_2\text{S}+\text{H}$).

Synthesis of 2-(E-5-Decenoylamino)ethyl Carbamoylmethyl Sulfide (I-1-g)
The procedures used for I-1-a were repeated with *E*-5-decenoic acid, affording I-1-g as a crystal powder, mp $130.5\text{--}131.5^\circ\text{C}$. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3380, 3320, 3180, 1640, 1540, 960. $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : 0.83–0.90 (3H, m), 1.16–1.36 (4H, m), 1.45–1.60 (2H, m), 1.88–2.09 (6H, m), 2.56–2.65 (2H, s), 3.08 (2H, s), 3.17–3.33 (2H, m), 5.24–5.50 (2H, m). HRMS m/z : 287.1819 [M+H] (287.1792 Calcd for $\text{C}_{14}\text{H}_{26}\text{N}_2\text{O}_2\text{S}+\text{H}$).

Synthesis of 2-(Z-5-Decenoylamino)ethyl Carbamoylmethyl Sulfide (I-1-h)
The procedures used for I-1-a were repeated with *Z*-5-decenoic acid,

affording I-1-h as a crystal powder, mp $115.0\text{--}115.5^\circ\text{C}$. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3380, 3320, 3180, 1640, 1540. $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : 0.83–0.90 (3H, m), 1.17–1.37 (4H, m), 1.44–1.59 (2H, m), 1.92–2.10 (6H, m), 2.55–2.65 (2H, s), 3.08 (2H, s), 3.17–3.33 (2H, m), 5.24–5.42 (2H, m). HRMS m/z : 287.1809 [M+H] (287.1792 Calcd for $\text{C}_{14}\text{H}_{26}\text{N}_2\text{O}_2\text{S}+\text{H}$).

Synthesis of 2-(E-6-Decenoylamino)ethyl Carbamoylmethyl Sulfide (I-1-i)
The procedures used for I-1-a were repeated with *E*-6-decenoic acid, affording I-1-i as a crystal powder, mp $135.5\text{--}137.0^\circ\text{C}$. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3370, 3310, 3180, 1645, 1635, 1540, 965. $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : 0.85 (3H, t, $J=7.4\text{ Hz}$), 1.23–1.55 (6H, m), 1.80–2.09 (6H, m), 2.57–2.64 (2H, m), 3.08 (2H, s), 3.17–3.27 (2H, m), 5.25–5.47 (2H, m). HRMS m/z : 287.1812 [M+H] (287.1792 Calcd for $\text{C}_{14}\text{H}_{26}\text{N}_2\text{O}_2\text{S}+\text{H}$).

Synthesis of 2-(Z-6-Decenoylamino)ethyl Carbamoylmethyl Sulfide (I-1-j)
The procedures used for I-1-a were repeated with *Z*-6-decenoic acid, affording I-1-j as a crystal powder, mp $115.5\text{--}117.0^\circ\text{C}$. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3370, 3310, 3180, 1645, 1635, 1545. $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : 0.86 (3H, t, $J=7.4\text{ Hz}$), 1.19–1.57 (6H, m), 1.92–2.10 (6H, m), 2.57–2.64 (2H, s), 3.08 (2H, s), 3.17–3.27 (2H, m), 5.25–5.41 (2H, m). HRMS m/z : 287.1791 [M+H] (287.1792 Calcd for $\text{C}_{14}\text{H}_{26}\text{N}_2\text{O}_2\text{S}+\text{H}$).

Synthesis of 2-(E-2-Decenoylamino)ethyl 4-Methyl-1-piperazinylcarbamoylmethyl Sulfide (II-1-a)
The procedures used for I-1-a were repeated with chloroacetic acid, affording 2-(*E*-2-decenoylamino)ethyl carboxymethyl sulfide. *N,N'*-Dicyclohexylcarbodiimide and *N*-methylpiperazine in dry chloroform was added dropwise to a stirred solution of 2-(*E*-2-decenoylamino)ethyl carboxymethyl sulfide in dry chloroform at 0°C , and stirred for 3.75 h at room temperature. The suspension was filtered, and the filtrate was evaporated. The residue was chromatographed on silica gel (93:7 chloroform-MeOH). The obtained crystal was crystallized from EtOAc/hexane (1:4), affording II-1-a as a needle-shaped white crystal, mp $77\text{--}78^\circ\text{C}$. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3280, 2920, 2770, 1665, 1620, 1550. $^1\text{H-NMR}$ (CDCl_3) δ : 0.88 (3H, brt), 1.15–1.55 (10H, m), 2.10–2.22 (2H, m), 2.32 (3H, s), 2.37–2.52 (4H, m), 2.75–2.85 (2H, m), 3.45–3.70 (6H, m), 5.83 (1H, dt, $J=15.3, 1.5\text{ Hz}$), 6.80 (1H, brt), 6.84 (1H, dt, $J=15.3, 7.0\text{ Hz}$). HRMS m/z : 370.2478 [M+H] (370.2526 Calcd for $\text{C}_{19}\text{H}_{35}\text{N}_3\text{O}_2\text{S}+\text{H}$).

Synthesis of 2-(E-2-Decenoylamino)ethyl 2-Cyclohexylethyl Sulfide (III-1-a)
The procedures used for I-1-a were repeated with 2-bromoethylcyclohexane, affording III-1-a as yellow paste. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3250, 2920, 1660, 1625, 1540. $^1\text{H-NMR}$ (CDCl_3) δ : 0.78–1.05 (5H, m), 1.06–1.55 (16H, m), 1.55–1.80 (5H, m), 2.10–2.25 (2H, m), 2.48–2.62 (2H, m), 2.69 (2H, t, $J=15.3, 1.4\text{ Hz}$), 5.92 (1H, brt), 6.84 (1H, dt, $J=15.3, 7.0\text{ Hz}$). HRMS m/z : 340.2684 [M+H] (340.2672 Calcd for $\text{C}_{20}\text{H}_{37}\text{NOS}+\text{H}$).

Synthesis of 2-(E-2-Decenoylamino)ethyl Cyclohexylmethyl Sulfide (IV-1-a)
The procedures used for I-1-a were repeated with iodomethylcyclohexane, affording IV-1-a as yellow paste. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3300, 1660, 1620, 1540. $^1\text{H-NMR}$ (CDCl_3) δ : 0.88 (3H, brt), 0.90–1.58 (16H, m), 1.58–1.91 (5H, m), 2.10–2.26 (2H, m), 2.42 (2H, d, $J=6.8\text{ Hz}$), 2.67 (2H, t, $J=6.5\text{ Hz}$), 3.50 (2H, q, $J=6.2\text{ Hz}$), 5.78 (1H, dt, $J=15.3, 1.5\text{ Hz}$), 5.97 (1H, brt), 6.84 (1H, dt, $J=15.3, 7.0\text{ Hz}$). HRMS m/z : 326.2494 [M+H] (326.2515 Calcd for $\text{C}_{19}\text{H}_{35}\text{NOS}+\text{H}$).

Synthesis of 2-(E-2-Decenoylamino)ethyl 2-Phenylethyl Sulfide (V-1-a)
The procedures used for I-1-a were repeated with 2-bromoethylbenzene, affording V-1-a as a needle-shaped white crystal, mp $53.0\text{--}53.3^\circ\text{C}$. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3280, 2910, 1660, 1620, 1540. $^1\text{H-NMR}$ (CDCl_3) δ : 0.88 (3H, brt), 1.15–1.55 (10H, m), 2.10–2.28 (2H, m), 2.69 (2H, t, $J=6.4\text{ Hz}$), 2.73–2.98 (4H, m), 3.49 (2H, q, $J=6.2\text{ Hz}$), 5.75 (1H, dt, $J=15.3, 1.4\text{ Hz}$), 5.89 (1H, brt), 6.83 (1H, dt, $J=15.3, 7.0\text{ Hz}$), 7.16–7.36 (5H, m). HRMS m/z : 334.2165 [M+H] (334.2203 Calcd for $\text{C}_{20}\text{H}_{31}\text{NOS}+\text{H}$).

Synthesis of 2-(E-2-Decenoylamino)ethyl Phenylmethyl Sulfide (VI-1-a)
The procedures used for I-1-a were repeated with benzyl bromide, affording VI-1-a as a needle-shaped white crystal, mp $52.0\text{--}52.5^\circ\text{C}$. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3300, 2920, 1665, 1625, 1545, 700. $^1\text{H-NMR}$ (CDCl_3) δ : 0.88 (3H, brt), 1.11–1.53 (10H, m), 2.00–2.21 (2H, m), 2.58 (2H, t, $J=6.4\text{ Hz}$), 3.44 (2H, q, $J=6, 2\text{ Hz}$), 3.71 (2H, s), 5.74 (1H, dt, $J=15.2, 1.4\text{ Hz}$), 6.05 (1H, brt), 6.81 (1H, dt, $J=15.3, 7.0\text{ Hz}$), 7.20–7.33 (5H, m). HRMS m/z : 320.2027 [M+H] (320.2047 Calcd for $\text{C}_{19}\text{H}_{33}\text{NOS}+\text{H}$).

Synthesis of 2-(E-2-Decenoylamino)ethyl *n*-Butyl Sulfide (VII-1-a)
The procedures used for I-1-a were repeated with *n*-butyl bromide, affording VII-1-a as a scaly-shaped white crystal, mp $48.0\text{--}48.5^\circ\text{C}$. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3220, 3070, 2920, 2850, 1660, 1620, 1550, 1465, 975. $^1\text{H-NMR}$ (CDCl_3) δ : 0.70–1.10 (6H, m), 1.10–1.90 (14H, m), 2.40–2.90 (4H, m). HRMS m/z : 286.2189 [M+H] (286.2203 Calcd for $\text{C}_{16}\text{H}_{31}\text{NOS}+\text{H}$).

Synthesis of 2-(E-2-Decenoylamino)ethyl Methyl Sulfide (VIII-1-a)
The procedures used for I-1-a were repeated with methyl iodide, affording VIII-1-a as a needle-shaped white crystal, mp $51.0\text{--}51.5^\circ\text{C}$. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} :

3300, 3090, 2930, 2860, 1665, 1630, 1545, 980. $^1\text{H-NMR}$ (CDCl_3) δ : 0.90 (3H, m), 1.08–1.80 (10H, brs), 2.10 (3H, s), 1.90–2.50 (2H, m), 2.65 (2H, t, $J=6$ Hz), 3.50 (2H, dt, $J=6$, 6 Hz), 5.82 (1H, dt, $J=15$, 1.3 Hz), 5.90–6.50 (1H, br t), 6.88 (1H, dt, $J=15$, 6.6 Hz). HRMS: m/z : 244.1694 $[\text{M}+\text{H}]$ (244.1734 Calcd for $\text{C}_{13}\text{H}_{25}\text{NOS}+\text{H}$).

Synthesis of 2-(E-2-Decenylamino)ethyl (1-Methyl-2-piperidyl)methyl Sulfide (IX-1-a) The procedures used for I-1-a were repeated with 1-methyl-2-chloromethylpiperidine, affording IX-1-a as yellow paste. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3300, 3090, 2920, 2870, 2800, 1665, 1630, 1540, 975, 755. $^1\text{H-NMR}$ (CDCl_3) δ : 1.10–2.00 (16H, m), 2.00–2.50 (6H, m), 2.50–3.10 (6H, m). HRMS m/z : 341.2642 $[\text{M}+\text{H}]$ (341.2625 Calcd for $\text{C}_{19}\text{H}_{36}\text{N}_2\text{OS}+\text{H}$).

The abbreviated name and structural formula of each synthesized compound is shown in Fig. 1.

Stress-Induced Gastric Ulcer Male Wistar rats weighing 230–250 g and fasted for 24 h were used as experimental animals. The rats were subjected to stress following the method of Takagi and Okabe,⁶⁾ in which animals were immobilized in a stress cage and immersed vertically in a water bath at $22 \pm 1^\circ\text{C}$ to the level of the xiphoid process. Each sample was suspended with 10% HCO-60[®] (polyoxyethylene harden castor oil; Nikko Chemicals) in saline and administered perorally, immediately or 1.5 h before the stress treatment. The animals were sacrificed 7 h later and their stomachs were removed, inflated with 10 ml of saline and immersed in 1% formalin solution for 5 min. The stomach was incised along the greater curvature and examined for gastric lesions. Total length (mm) of all lesions in the glandular portion of the stomach was used as an ulcer index.

abbreviated name	structural formula	
I-1-a		
I-2-a	a	2: -CH ₂ - I
I-3-a	a	3: -O- I
I-1-b	b:	1 I
I-1-c	c:	1 I
I-1-d	d:	1 I
I-1-e	e:	1 I
I-1-f	f:	1 I
I-1-g	g:	1 I
I-1-h	h:	1 I
I-1-i	i:	1 I
I-1-j	j:	1 I
II-1-a	a	1 II: -CH ₂ -CO-N-CH ₃
III-1-a	a	1 III: -CH ₂ -CH
IV-1-a	a	1 IV: -CH
V-1-a	a	1 V: -CH
VI-1-a	a	1 VI: -CH
VII-1-a	a	1 VII: -CH
VIII-1-a	a	1 VIII: -CH
IX-1-a	a	1 IX: -CH

Fig. 1. The Abbreviated Name and Structural Formula of Each Synthesized Compound

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. Each sample, suspended in 10% HCO-60 in saline, was administered at doses of 500, 2000, 8000 mg/kg perorally at a volume of 40 ml/kg. After the administration of a single dose of each test sample, the behavior of the animals was observed for 6 h, then they were caged and fed as usual for 7 days. Number of dead animals and the change of body weight in each mouse were observed every day until the end of the experiment.

Statistics Results were expressed as the mean $\pm$ 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

The inhibitory effects of I-2-a and I-3-a replacing sulfur atom by methylene group and oxygen atom given at doses of 2 and 5 mg/kg, *p.o.*, on stress-induced ulceration in rats were compared with that of I-1-a. I-2-a and I-3-a showed significant anti-ulcerogenic activity, but their potencies were less than that of I-1-a (Table I).

The effect of modification of unsaturation in decenyl chain on stress-induced ulceration was tested. At doses of 5 and 2 mg/kg perorally immediately before the stress treatment, I-1-b with saturated fatty acid showed somewhat less pharmacological activity than I-1-a with unsaturated fatty acid (Table II).

The effect of position and configuration of unsaturation in various 2-(*E-n* or *Z-n*-decenylamino)ethyl carbamoyl-methyl sulfides, given at a dose of 5 mg/kg, *p.o.* is shown in Fig. 2. I-1-c, -e and -g retained inhibitory potency comparable to I-1-a and I-1-d, -f and -j almost completely lost it. There was no relationship between the position of

TABLE I. Effect of I-1-a, I-2-a and I-3-a on Stress-Induced Ulceration in Rats

Treatment	Dose (mg/kg)	Ulcer index	Inhibition (%)
Control ^{a)}	—	25.7 $\pm$ 4.2	—
I-1-a	5	8.2 $\pm$ 1.3 ^{c)}	68.1
	2	13.0 $\pm$ 3.1 ^{b)}	49.4
I-2-a	5	16.2 $\pm$ 2.0	37.0
	2	17.2 $\pm$ 3.0	33.1
Control ^{a)}	—	24.1 $\pm$ 3.0	—
I-1-a	5	9.6 $\pm$ 2.8 ^{c)}	60.1
	2	10.4 $\pm$ 2.1 ^{c)}	56.4
I-3-a	5	12.7 $\pm$ 2.3 ^{c)}	47.3
	2	19.1 $\pm$ 3.7	20.8

All values represent the mean $\pm$ 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) $p < 0.01$.

TABLE II. Effect of I-1-a and I-1-b on Stress-Induced Ulceration in Rats

Treatment	Dose (mg/kg)	Ulcer index	Inhibition (%)
Control ^{a)}	—	17.2 $\pm$ 2.2	—
I-1-a	5	4.3 $\pm$ 0.7 ^{d)}	75.0
	2	7.9 $\pm$ 0.8 ^{c)}	54.1
I-1-b	5	9.1 $\pm$ 0.8 ^{c)}	47.1
	2	11.4 $\pm$ 1.7 ^{b)}	33.1

All values represent the mean $\pm$ 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. Effect of Position and Configuration of Unsaturation in Various 2-(*E*-*n* or *Z*-*n*-Decenoylamino)ethyl Carbamoylmethyl Sulfides on Stress-Induced Ulceration in Rats

Each column represents relative percent inhibition of ulcer index in various 2-(*E*-*n* or *Z*-*n*-decenoylamino)ethyl carbamoylmethyl sulfides against that in the control group. The inhibitory activity of I-1-a was considered to 100%. Each sample was administered perorally (5mg/kg) immediately before the restraint and water-immersion stress loading.

Fig. 3. Effect of Alkyl Group in Various 2-(*E*-2-Decenoylamino)ethyl Alkyl Sulfides on Stress-Induced Ulceration in Rats

Each column represents relative percent inhibition of ulcer index in various 2-(*E*-2-decenoylamino)ethyl alkyl sulfides against that in the control group. The inhibitory activity of I-1-a was considered to 100%. Each sample was administered perorally (equimolar dose of 5mg/kg of I-1-a) 1.5h before the restraint and water-immersion stress loading.

unsaturation and the pharmacological activity. On the other hand, compounds with *E*-configuration showed stronger pharmacological activity than the corresponding *Z*-type compounds.

The effect of S-alkyl group in various 2-(*E*-2-decenoylamino)ethyl alkyl sulfides, given at an equimolar dose of 5 mg/kg of I-1-a perorally 1.5h before the stress treatment, was examined. As shown in Fig. 3, III-1-a with cyclohexylethyl group and IX-1-a with (1-methyl-2-piperidyl)-methyl group showed highly improved anti-ulcerogenic activity over I-1-a. The ulcer indices of control, I-1-a and III-1-a were 22.0 ± 3.6 , 12.8 ± 1.6 ($p < 0.05$) and 6.7 ± 1.7 ($p < 0.01$), respectively. The other analogues with different S-alkyl groups were less active than I-1-a.

TABLE III. Effect of Alkyl Group in Various 2-(*E*-2-Decenoylamino)ethyl Alkyl Sulfides on Acute Toxicity in Mice

Treatment	Dose (mg/kg)	Mortality	Toxic symptom
I-1-a	8000	0/5	Not observed
	2000	0/5	Not observed
	500	0/5	Not observed
II-1-a	2000	0/5	Mild
	500	0/5	Mild
III-1-a	2000	0/5	Not observed
	500	0/5	Not observed
IV-1-a	2000	0/5	Not observed
	500	0/5	Not observed
V-1-a	2000	0/5	Serious
	500	0/5	Not observed
VI-1-a	2000	0/5	Not observed
	500	0/5	Not observed
VII-1-a	200	0/5	Not observed
	500	0/5	Not observed
VIII-1-a	2000	0/5	Serious
	500	5/5	Severe
IX-1-a	2000	5/5	Severe
	500	5/5	Severe

Each sample, suspended in 10% HCO-60 in saline, was administered perorally at a volume of 40 ml/kg.

The acute toxicities of I-1-a—IX-1-a were tested in mice. No death or toxic symptoms were observed in mice given I-1-a, III-1-a, IV-1-a or VI-1-a at any examined doses (*p.o.*) during the experimental periods (Table III). IX-1-a, however, showed serious toxicity and mortality was 5/5.

Discussion

We have reported that several sulfur-containing amino acids and amines, especially cystamine, showed strong gastric secretion inhibitory activity.⁵⁾ Further, we found that in a series of 2-(*E*-2-alkenoylamino)ethyl alkyl sulfides which were synthesized from cystamine and various *E*-2-unsaturated fatty acids, I-1-a showed the most potent anti-ulcerogenic activity.⁵⁾ Recently, it has been noted that sulfur-containing compounds show multi-pharmacological activities such as radio-preventive,⁷⁾ cytoprotective,⁸⁾ and anti-rheumatoid⁹⁾ effects. To elucidate the necessity of sulfide in I-1-a, compounds replacing the sulfur atom by methylene group or oxygen atom were synthesized and their anti-ulcerogenic activities were tested. I-2-a and I-3-a showed reduced pharmacological activity, but did not lose it. It seems that sulfide linkage is not essential but is more favorable than methylene linkage or other linkage for showing the pharmacological activity, probably due to subtle differences of steric interactions among the atoms around the sulfur.

From the relationship studies between alkyl or alkenyl chain lengths and anti-ulcerogenic activity of various saturated and unsaturated fatty acids, we found *E*-2-decenoic acid to be the most effective.⁴⁾ Further, we discovered I-1-a to be the strongest anti-ulcerogenic compound in a series of hybrids which were synthesized from various *E*-2-unsaturated fatty acids and cystamine.⁵⁾ These results led us also to prepare analogues with modifications of unsaturation in the decenoyl chain. Saturation of the decenoyl chain resulted in some degree of potency loss. It seems likely that unsaturation relates to intestinal absorption rather than to action mechanism of

the compounds, because the saturated compound retains significant potency. There was no relationship between the position of unsaturation and the potency, while the configuration of unsaturation was important to the appearance of anti-ulcerogenic activity. Generally, most naturally occurring unsaturated fatty acids are *Z*-configuration. It is reported that many *Z*-configured fatty acids in natural products change to *E*-type in processing to foods,¹⁰⁾ and that the *E*-type fatty acids are absorbed from the digestive tract and oxidized *in vivo* as well as *Z*-type fatty acids.¹¹⁾ In the case of fatty acid alone, *Z*- and *E*-configured fatty acids are absorbed to a similar extent. On the other hand, fatty acid moiety in the derivatives of I-1-a probably related to the absorption rate of the compounds, as indicated by the result that *E*-configuration was essential to the appearance of the anti-ulcerogenic activity.

I-1-a with carbamoylmethyl as S-alkyl group is insoluble in water and many organic solvents, indicating its low bioavailability *in vivo*. Attempts have been made to increase the potency of I-1-a by replacing the carbamoylmethyl group with appropriate S-alkyl groups. Two compounds replacing S-alkyl group, III-1-a and IX-1-a were selected for more being potent inhibitors of ulceration than I-1-a.

Since no toxic symptom was observed at any tested dose in mice, III-1-a may be practical and useful as an anti-ulceration drug, but this requires further studies on its

action mechanism, metabolism and so on.

Acknowledgements We are grateful to Mr. Satoru Inoue and Mr. Tadashi Okano for their technical assistance.

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