INHIBITORY EFFECTS OF UNSATURATED FATTY ACIDS OF *trans*-2-C_{10:1} TO *trans*-2-C_{16:1} SEVERAL C_{18:n} AND C_{20:n} ON GASTRIC SECRETION AND EXPERIMENTAL ULCERATION IN RATS*

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Among various unsaturated fatty acids which were examined in this experiment, *trans*-2-tridecenoic acid was found to be a potent inhibitor on gastric secretion in rats (*i.p.* or *i.d.*). Therefore, the effects of *trans*-2-tridecenoic acid on several experimental ulcerations in rats were examined. At the dose of 10 mg/kg, *trans*-2-tridecenoic acid significantly decreased the ulcer index in pylorus-ligated rats; aspirin-, phenylbutazone-, histamine- and stress-induced ulcerations were also significantly inhibited at the dose of 25 mg/kg. Finally, effects of *trans*-2-tridecenoic acid on hexosamine and sialic acid contents in the gastric mucous were examined. In intact or phenylbutazone-induced ulcerated rats, *trans*-2-tridecenoic acid increased the sialic acid levels in the gastric mucous at the dose of 25 mg/kg. The decreased hexosamine content in the gastric mucous by phenylbutazone treatment was recovered by the administration of *trans*-2-tridecenoic acid.

Keywords — unsaturated fatty acid; *trans*-2-tridecenoic acid; gastric juice secretion; pylorus-ligated ulcer; aspirin-induced ulcer; phenylbutazone-induced ulcer; histamine-induced ulcer; stress-induced ulcer; mucosal hexosamine; mucosal sialic acid

We reported earlier the isolation of several fatty acids from dried brewer's yeast as an active principle inhibiting gastric juice secretion in rats.¹⁾ In pursuing the structure-activity correlation of saturated fatty acids, we examined the inhibitory activities of normal saturated fatty acids of C_{10:0} to C_{20:0} and branched saturated fatty acids of iso-C_{12:0} to iso-C_{16:0} on gastric secretion in rats. Myristic acid and iso-C_{15:0} were found to have the strongest gastric acid secretion inhibitory activity in rats among normal acids²⁾ and branched chain fatty acids,³⁾ respectively.

In this paper, we examined the inhibitory activities of unsaturated fatty acids of *trans*-2-C_{10:1} to *trans*-2-C_{16:1}, several C_{18:n} and C_{20:n} on gastric juice secretion in rats. Examinations were also made on the effect of *trans*-2-

tridecenoic acid (C_{13:1}), which had the most marked activity, on various experimental ulcer models and carbohydrate contents in gastric mucous.

MATERIALS AND METHODS

Materials — *trans*-2-Decenoic acid (C_{10:1}), *trans*-2-undecenoic acid (C_{11:1}), *trans*-2-tridecenoic acid (C_{13:1}), *trans*-2-hexadecenoic acid (C_{16:1}), *cis*-9-vaccenoic acid (C_{18:1}), *cis*-9, 12-linoleic acid (C_{18:2}) and *cis*-9, 12, 15-linolenic acid (C_{18:3}) were obtained from Tokyo Kasei Co., Japan. For the above fatty acids, 95, 89, 91, 99, 90, 90, 97, 95 and 73% purities were guaranteed by Tokyo Kasei using gas liquid chromatography (GLC). *cis*-6-Petroselinic acid (C_{18:1}), *trans*-9, 12-linoelaidic acid (C_{18:2}), *cis*-6, 9, 12-

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γ -linolenic acid ($C_{18:3}$), (Z)-11, 14-eicosadienoic acid ($C_{20:2}$) and (Z)-5, 8, 11, 14-arachidonic acid ($C_{20:4}$) were obtained from P.L. Biochemical Inc. (Z)-11-Eicosaenoic acid ($C_{20:1}$) and (Z)-5, 8, 11, 17-eicosapentaenoic acid ($C_{20:5}$) were obtained from Serdary Research Laboratories. These fatty acids were guaranteed to be 99% pure by both GLC and thin layer chromatography (TLC). *trans*-2-Dodecenoic acid ($C_{12:1}$), *trans*-2-tetradecenoic acid ($C_{14:1}$) and *trans*-2-pentadecenoic acid ($C_{15:1}$) were synthesized as described below. These fatty acids showed more than 99% purity by GLC.

Preparation of Olefinic Acid Homologs — i) *trans*-2-Pentadecenoic Acid: *n*-Tridecylaldehyde (8.2 g) was condensed at 95–100°C with dry malonic acid (5.2 g) according to the Doebner procedure,⁴⁾ using dry pyridine (8 ml) containing piperidine (0.1 ml). The reaction mixture was poured into ice-cooled 10% sulfuric acid (120 ml), and the colorless precipitates were collected, washed with water, and separated by suction into a colorless solid (5.0 g) and a colorless oil (4.1 g). Fractional distillation of the solid and oil under reduced pressure gave a total of 50.3% yield (5.0 g) of the title acid as a colorless crystalline mass, bp 168–170°C (4.5 mmHg).

ii) *trans*-2-Tetradecenoic Acid: *n*-Dodecylaldehyde (8.4 g) was prepared by the pyridinium chlorochromate (PCC) oxidation⁵⁾ of *n*-dodecylalcohol and was condensed with malonic acid (7.1 g) in dry pyridine (15 ml) containing piperidine (0.1 ml) to give the title compound (6.3 g, 61.4% of the theoretical) as a colorless oil, bp 161–164°C (5 mmHg), which became a solid colorless crystalline mass.

iii) *trans*-2-Dodecenoic Acid: The Doebner condensation⁴⁾ of *n*-decylaldehyde (21.2 g) with malonic acid (17.9 g) in dry pyridine (28 ml) containing piperidine (0.1 ml) gave the title compound (10.0 g, 37.2 of the theoretical) as a colorless oil, bp 150–150.5°C (4.5 mmHg).

The structures of the olefinic acids prepared were determined by infrared, ^1H - and ^{13}C -nuclear magnetic resonance, and gas chromatography-mass spectrometric measurement.

Atropine sulfate was obtained from Wako Chemical Industries, Ltd. Cimetidine was kindly supplied by Smith Klein & Fujisawa.

Assay of Gastric Juice Secretion Inhibitory Activity in Rats — The assay was performed as follows. Male Wistar rats weighing 150–200 g, fasted for 48 h, were anesthetized with ethyl ether and then pylorus-ligated. Each sample was suspended in 5% Gum Arabic solution and administered intraperitoneally or intraduodenally immediately after pylorus-ligation. Four h after the pylorus-ligation, the inner contents of the stomach were collected and the volume of gastric juice and total peptic activity were determined by the methods described in our previous paper.⁶⁾

Capillary Permeability Test in Mice — Male mice (ddy strain) weighing 20 ± 1 g, were given an intravenous injection of 0.1 ml of 4% Pontamine Sky Blue 6BX according to the method described by Whittle.⁷⁾ After 10 min, *trans*-2-tridecenoic acid or 1% acetic acid was administered intraperitoneally. After 20 min, the mice were killed by dislocation of the neck and the viscera were exposed. Each animal was held by the flap of the abdominal wall and the peritoneal cavity was washed with distilled water over a petri dish. The optical density of the solution was read at 590 nm.

Experimental Gastric Ulcers in Rats — i) Gastric Ulceration in Pylorus-Ligated Rats: The anti-ulcerogenic activity in pylorus-ligated rats was measured as follows. Male Wistar rats weighing 150–200 g, fasted for 24 h, were ligated in the pylorus and each sample was intraperitoneally administered at the same dose twice, immediately and 8 h after the pylorus-ligation. After 16 h, the rats were sacrificed and the stomachs removed. The degree of gastric ulceration was estimated by the method of Narumi *et al.*⁸⁾ Cimetidine was used as a positive control reagent.

ii) Aspirin-Induced Gastric Ulcer: Male Wistar rats weighing 200–250 g were deprived of food for 24 h. According to the method of

Okabe *et al.*,⁹⁾ rats orally received 100 mg/kg of aspirin suspended in 5% Gum Arabic solution immediately after the pylorus-ligation. Each test sample was administered intraperitoneally concurrently with the pylorus-ligation. Cimetidine was used as a positive control reagent. Seven h after the pylorus-ligation, rats were sacrificed and their stomachs removed to examine the lesion in the glandular portion of the stomach. The sum of the lengths of all lesions was used as an ulcer index.

iii) Phenylbutazone-Induced Gastric Ulcer: This ulceration model was induced according to the method of Suzuki *et al.*¹⁰⁾ Male Wistar rats weighing 200–250 g were fasted for 24 h, but were provided with water *ad libitum*. Each rat received 200 mg/kg of phenylbutazone suspended in 5% Gum Arabic solution orally. Rats, deprived of solid food and water for 5 h, were sacrificed and their stomachs removed to examine the lesion in the glandular stomach area. The sum of the diameter of each lesion was used as an ulcer index. A test sample was administered intraperitoneally 30 min before the administration of phenylbutazone. Atropine sulfate was used as a positive control reagent.

iv) Histamine-Induced Ulceration: Male Wistar rats weighing 150–200 g were fasted for 48 h, but were provided with water *ad libitum*. This ulcer model was induced according to the method of Buchner *et al.*¹¹⁾ Each rat was administered 300 mg/kg of histamine hydrochloride intraperitoneally. Rats, deprived of solid food and water for 4 h, were sacrificed and their stomachs removed to examine the lesion in the glandular portion. The ulcer index was estimated by the method of Adami *et al.*¹²⁾ Each sample was administered intraperitoneally 10 min before the administration of histamine hydrochloride. Cimetidine was used as a positive control reagent.

v) Stress-Induced Gastric Ulcer: Male Wistar rats weighing 200–250 g were used as experimental animals. Rats were subjected to stress following the method of Takagi *et al.*¹³⁾ in which animals were immobilized in the stress cage and

immersed in a water bath at 23°C to the depth of the xiphoid for 20 h. Each sample was administered intraperitoneally twice, 15 min prior and 10 h after the stress treatment. Cimetidine was also administered to the positive control group in the same manner. The animals were sacrificed 20 h after being subjected to stress and the stomachs removed and checked in order to measure the ulcer area and obtain an ulcer index.

Effect of trans-2-Tridecenoic Acid on Stomach Perfusion Preparation — The effect of *trans*-2-tridecenoic acid on stimulated gastric juice secretion by various kinds of drugs was examined by Sawada's modification¹⁶⁾ of the Ghosh and Schild stomach perfusion method.¹⁷⁾ Male Wistar rats weighing 250–300 g were fasted for 48 h, then anesthetized with urethane. A perfusion loop to the stomach was made by catheterization. After washing the stomach, physiological saline maintained at $30 \pm 1^\circ\text{C}$ was perfused (at the speed of 1 ml/min) to the stomach and the perfusate was collected every 10 min by the fraction collector. Ten micrograms per kilogram of tetragastrin (Nissui Seiyaku Co.) and 1 $\mu\text{g/kg}$ each of histamine (Nakarai Chem., Ltd.) or carbachol (Sigma Chem. Co.) were administered intravenously through the femoral vein. Twenty-five milligrams per kilogram of *trans*-2-tridecenoic acid was administered intraperitoneally 60 min before the second injection of the stimulants. The acidity of each fraction was titrated with 1/200 N NaOH solution by using phenolphthalein as an indicator.

Measurement of Hexosamine and Sialic Acid Contents in Gastric Mucous — Phenylbutazone-induced ulceration was produced according to the method described above. Then phenylbutazone-induced ulcerated or intact rats were sacrificed by a blow on the head. The stomachs were removed quickly and incised along the greater curvature. Each stomach was washed lightly with saline, and mucosal tissue from the glandular portion was scraped off and weighed.

i) Determination of Hexosamine: Each mucous was homogenized and digested with 3 ml of papain solution (Difco; 100 units of

papain in 100 ml of 0.2 M acetate buffer at pH 5.6 containing 2 mM EDTA, 4 mM cystein, 0.88% NaCl) for 20 h at 37°C, and centrifuged to eliminate indigestive components. A portion of the supernatant was hydrolyzed in 2 N HCl in a sealed ampoule at 110°C for 14 h. Hexosamine was determined by the colorimetric method of Gunnar,¹⁴ and calculated as glucosamine hydrochloride.

ii) Determination of Sialic Acid: After 1 h hydrolysis of the homogenate of the scraped mucous in 0.1 N H₂SO₄ at 80°C, sialic acid content was determined by the method of thiobarbituric acid,¹⁵ using *N*-acetylneuraminic acid as a standard.

The Effect of trans-2-Tridecenoic Acid on the Gastric Emptying Rate in Rats — Male Wistar rats weighing 150–200 g were fasted for 48 h. The effect of *trans*-2-tridecenoic acid on the gastric emptying rate was examined by the method of Scarpignato *et al.*¹⁸ Each rat received 1.5 ml of the pre-warmed (35°C) test meal orally. The sample was administered intraperitoneally 40 min before the administration of the test meal. The animals were killed by cervical dislocation at fixed time intervals (20 min) after incubation. Control animals were killed immediately after administration of the test meal. A solution of 50 mg phenol red in 100 ml aqueous methylcellulose (1.5%) was used as a test meal. The stomach was then exposed by laparotomy, quickly ligated at the pylorus and the cardia, and removed. Assay procedure of phenol red represented a modification of the method described by Reynell *et al.*¹⁰ The gastric emptying rate for each rat was calculated according to the following formula:

$$= \left[1 - \frac{\text{Amount of phenol red recovered from the test stomach}}{\text{Average amount of phenol red recovered from the control stomachs}} \right] \times 100$$

RESULTS

Inhibitory Effects of Unsaturated Fatty Acids of trans-2-C_{10:1} to C_{16:1}, Several C_{18:n} and C_{20:n} on

Gastric Secretion in Rats

Inhibitory effects of *trans*-2-C_{10:1} to C_{16:1} on gastric juice secretion in rats were examined in pylorus-ligated rats. As shown in Table I, at the dose of 25 mg/kg *i.p.*, all of the fatty acids C_{10:1} to C_{16:1} inhibited significantly the volume of gastric juice, total acid output and total peptic activity. At the dose of 10 mg/kg *i.p.*, *trans*-2-tridecenoic acid (C_{13:1}) and *trans*-2-hexadecenoic acid (C_{16:1}) inhibited significantly these three parameters, whereas *trans*-2-tetradecenoic acid (C_{14:1}) and *trans*-2-pentadecenoic acid (C_{15:1}) inhibited only gastric volume and total acid output. When the dose was decreased to 5 mg/kg *i.p.*, only *trans*-2-tridecenoic acid (C_{13:1}) showed inhibitory activity in the three parameters. At the dose of 2.5 mg/kg *i.p.*, *trans*-2-tridecenoic acid had no effect on gastric secretion in rats, as shown in Table II. Moreover, neither C_{18:n} nor C_{20:n} showed any significant inhibitory effects at 5 mg/kg *i.p.*, as shown in Tables III and IV.

It is widely accepted that the gastric serosal membrane irritating substances, such as acetic acid or surfactant, have a gastric secretion inhibitory activity when administered intraperitoneally and showed little effect when administered intraduodenally. Therefore, the gastric secretion inhibitory activity of the intraduodenally administered *trans*-2-tridecenoic acid was examined.

As shown in Table V, at the dose of 10 mg/kg *i.d.*, *trans*-2-tridecenoic acid significantly inhibited gastric juice volume, total acid output and total peptic activity, but at 5 mg/kg *i.d.*, *trans*-2-tridecenoic acid did not show any significant inhibition of these three parameters.

Capillary Permeability Test of trans-2-Tridecenoic Acid in Mice

trans-2-Tridecenoic acid had no irritation effect as a surfactant at the dose of 25 mg/kg *i.p.* PSB-6BX which emerged into the peritoneal cavity was measured to be 4.22 µg/ml in the control group and 4.40 µg/ml in the *trans*-2-tridecenoic acid (25 mg/kg *i.p.*) treated group.

Anti-ulcerogenic Activity of trans-2-Tridecenoic

Acid in Rats

The anti-ulcerogenic activities of *trans*-2-

tridecenoic acid were examined in the following experimental ulcer models.

TABLE I. *Effects of Various Fatty Acids on Gastric Secretion in Pylorus-Ligated Rats (4 h)*

Treatment	Dose (mg/kg)	Gastric volume (ml/100g b.w.)	Total acid output (μ eq/100g b.w.)	Total peptic activity (mg as tyrosine/100g b.w.)
Control ^{a)}	—	3.12 $\pm$ 0.26	296.4 $\pm$ 28.8	215.6 $\pm$ 13.8
Decenoic acid (C _{10:1})	25	1.29 $\pm$ 0.16***	75.9 $\pm$ 12.8***	73.8 $\pm$ 7.5***
Undecenoic acid (C _{11:1})	25	1.97 $\pm$ 0.29*	136.8 $\pm$ 17.4**	117.4 $\pm$ 19.6**
Dodecenoic acid (C _{12:1})	25	1.74 $\pm$ 0.12***	154.4 $\pm$ 9.7***	132.0 $\pm$ 7.1***
Tridecenoic acid (C _{13:1})	25	1.24 $\pm$ 0.11***	95.4 $\pm$ 10.1***	107.2 $\pm$ 6.2***
Tetradecenoic acid (C _{14:1})	25	1.56 $\pm$ 0.16***	131.8 $\pm$ 18.1***	117.0 $\pm$ 10.8***
Pentadecenoic acid (C _{15:1})	25	1.52 $\pm$ 0.15***	131.2 $\pm$ 16.6***	106.8 $\pm$ 10.7***
Hexadecenoic acid (C _{16:1})	25	1.26 $\pm$ 0.11***	88.2 $\pm$ 9.2***	92.8 $\pm$ 6.2***
Control ^{a)}	—	3.22 $\pm$ 0.33	224.0 $\pm$ 29.9	250.8 $\pm$ 29.1
Decenoic acid (C _{10:1})	10	2.80 $\pm$ 0.50	211.6 $\pm$ 25.8	186.0 $\pm$ 32.6
Undecenoic acid (C _{11:1})	10	3.10 $\pm$ 0.45	220.0 $\pm$ 20.2	204.7 $\pm$ 25.7
Dodecenoic acid (C _{12:1})	10	2.45 $\pm$ 0.54	166.6 $\pm$ 42.0	231.7 $\pm$ 38.2
Tridecenoic acid (C _{13:1})	10	2.20 $\pm$ 0.23*	113.8 $\pm$ 21.9*	162.5 $\pm$ 18.7*
Tetradecenoic acid (C _{14:1})	10	1.94 $\pm$ 0.29*	124.7 $\pm$ 15.5*	182.9 $\pm$ 14.2
Pentadecenoic acid (C _{15:1})	10	1.88 $\pm$ 0.31*	121.5 $\pm$ 17.5*	188.5 $\pm$ 15.2
Hexadecenoic acid (C _{16:1})	10	1.24 $\pm$ 0.35**	85.1 $\pm$ 15.4**	133.1 $\pm$ 17.4**
Control ^{a)}	—	2.88 $\pm$ 0.45	323.2 $\pm$ 66.3	233.6 $\pm$ 23.5
Atropine sulfate	5	0.29 $\pm$ 0.06***	28.9 $\pm$ 4.9***	35.2 $\pm$ 5.0***
	1	0.83 $\pm$ 0.16***	75.5 $\pm$ 10.0**	79.0 $\pm$ 11.0***

All values represent mean $\pm$ s.e. (n=8).

a) 5% Acacia in saline.

Each sample was administered immediately after pylorus ligation (i.p.).

Significantly different from the control group: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

TABLE II. *Effects of Various Fatty Acids on Gastric Secretion in Pylorus-Ligated Rats (4 h)*

Treatment	Dose (mg/kg)	Gastric volume (ml/100g b.w.)	Total acid output (μ eq/100g b.w.)	Total peptic activity (mg as tyrosine/100g b.w.)
Control ^{a)}	—	3.22 $\pm$ 0.34	344.6 $\pm$ 46.8	283.0 $\pm$ 32.8
Dodecenoic acid (C _{12:1})	5	3.64 $\pm$ 0.34	384.2 $\pm$ 34.2	306.8 $\pm$ 26.6
Tridecenoic acid (C _{13:1})	5	1.88 $\pm$ 0.36*	155.4 $\pm$ 43.0*	166.8 $\pm$ 25.0*
	2.5	2.51 $\pm$ 0.27	223.8 $\pm$ 25.1	277.6 $\pm$ 11.2
Tetradecenoic acid (C _{14:1})	5	2.64 $\pm$ 0.46	288.0 $\pm$ 53.6	203.8 $\pm$ 33.8
Pentadecenoic acid (C _{15:1})	5	3.88 $\pm$ 0.30	435.8 $\pm$ 31.0	304.0 $\pm$ 23.2
Hexadecenoic acid (C _{16:1})	5	3.68 $\pm$ 0.38	402.8 $\pm$ 55.6	319.6 $\pm$ 40.4

All values represent mean $\pm$ s.e. (n=8).

a) 5% Acacia in saline.

Each sample was administered immediately after pylorus ligation (i.p.).

Significantly different from the control group: * $p < 0.05$.

As shown in Table VI, *trans*-2-tridecenoic acid significantly decreased the ulcer index in pylorus-ligated rats at the dose of 10 mg/kg *i.p.* When the dose was decreased to 5.0 mg/kg,

TABLE III. *Effects of Various Fatty Acids on Gastric Secretion in Pylorus-Ligated Rats (4 h)*

Treatment	Dose (mg/kg)	Gastric volume (ml/100g b.w.)	Total acid output (μ eq/100g b.w.)	Total peptic activity (mg as tyrosine/100g b.w.)
Control ^{a)}	—	2.37 $\pm$ 0.21	230.1 $\pm$ 27.4	242.2 $\pm$ 10.6
Oleic acid (C _{18:1})	25	1.62 $\pm$ 0.23*	128.4 $\pm$ 17.9**	194.8 $\pm$ 11.4
Vaccenic acid (C _{18:1})	25	1.39 $\pm$ 0.13**	129.2 $\pm$ 13.0**	172.0 $\pm$ 6.1*
Petroselinic acid (C _{18:1})	25	1.76 $\pm$ 0.24	133.9 $\pm$ 39.4	128.2 $\pm$ 19.0*
Elaidic acid (C _{18:1})	25	1.65 $\pm$ 0.11*	142.2 $\pm$ 14.8*	158.4 $\pm$ 6.6*
Linolic acid (C _{18:2})	25	1.67 $\pm$ 0.13*	165.8 $\pm$ 15.6	194.8 $\pm$ 8.3
Linoelaidic acid (C _{18:2})	25	2.57 $\pm$ 0.42	212.5 $\pm$ 39.8	211.8 $\pm$ 14.8
Linolenic acid (C _{18:3})	25	1.81 $\pm$ 0.19	149.9 $\pm$ 18.9*	175.8 $\pm$ 10.0*
γ -Linolenic acid (C _{18:3})	25	3.24 $\pm$ 0.37	284.7 $\pm$ 47.4	255.6 $\pm$ 16.9
Control ^{a)}	—	3.42 $\pm$ 0.48	253.4 $\pm$ 44.0	231.2 $\pm$ 25.2
Oleic acid (C _{18:1})	10	2.16 $\pm$ 0.64	114.4 $\pm$ 17.8	175.2 $\pm$ 9.8
	5	2.74 $\pm$ 0.42	220.8 $\pm$ 26.2	208.3 $\pm$ 19.2
Vaccenic acid (C _{18:1})	10	1.46 $\pm$ 0.26**	98.2 $\pm$ 19.2**	131.3 $\pm$ 13.8**
	5	2.44 $\pm$ 0.33	168.5 $\pm$ 29.9	188.5 $\pm$ 20.3
Elaidic acid (C _{18:1})	10	1.76 $\pm$ 0.34*	128.6 $\pm$ 23.8*	123.2 $\pm$ 16.2**
	5	2.92 $\pm$ 0.43	227.0 $\pm$ 29.3	239.2 $\pm$ 15.6
Linolic acid (C _{18:2})	10	2.06 $\pm$ 0.34*	144.4 $\pm$ 30.2	150.0 $\pm$ 18.6*
	5	3.25 $\pm$ 0.28	210.8 $\pm$ 27.4	232.4 $\pm$ 15.7

All values represent mean $\pm$ s.e. (n=8).

a) 5% Acacia in saline.

Each sample was administered immediately after pylorus ligation (*i.p.*).

Significantly different from the control group: * $p < 0.05$, ** $p < 0.01$.

TABLE IV. *Effects of Various Fatty Acids on Gastric Secretion in Pylorus-Ligated Rats (4 h)*

Treatment	Dose (mg/kg)	Gastric volume (ml/100g b.w.)	Total acid output (μ eq/100g b.w.)	Total peptic activity (mg as tyrosine/100g b.w.)
Control ^{a)}	—	2.90 $\pm$ 0.22	157.6 $\pm$ 23.0	222.8 $\pm$ 25.5
Eicosenoic acid (C _{20:1})	25	2.31 $\pm$ 0.29	114.6 $\pm$ 18.2	136.2 $\pm$ 25.4
Eicosadienoic acid (C _{20:2})	25	2.30 $\pm$ 0.40	107.4 $\pm$ 19.8	163.0 $\pm$ 27.8
Arachidonic acid (C _{20:4})	25	1.66 $\pm$ 0.37*	78.1 $\pm$ 11.4**	113.0 $\pm$ 15.4**
	10	2.28 $\pm$ 0.52	113.1 $\pm$ 17.2	185.2 $\pm$ 28.4
	5	3.10 $\pm$ 0.54	145.0 $\pm$ 22.2	204.2 $\pm$ 33.0
Eicosapentaenoic acid (C _{20:5})	25	3.11 $\pm$ 0.40	180.6 $\pm$ 26.0	223.9 $\pm$ 32.3
	10	2.49 $\pm$ 0.40	157.6 $\pm$ 24.0	217.5 $\pm$ 25.0
	5	2.66 $\pm$ 0.46	169.9 $\pm$ 19.2	179.6 $\pm$ 17.8

All values represent mean $\pm$ s.e. (n=8).

a) 5% Acacia in saline.

Each sample was administered immediately after pylorus ligation (*i.p.*).

Significantly different from the control group: * $p < 0.05$, ** $p < 0.01$.

trans-2-tridecenoic acid did not show any significant decrease. Cimetidine, used as a positive control, significantly decreased the ulcer index at the dose of 100 mg/kg *i.p.* *trans*-2-Tridecenoic acid also significantly prevented aspirin-, phenylbutazone-, histamine-, and stress-induced ulcerations at a dose of 25 mg/kg *i.p.* However, at a dose of 10 mg/kg *i.p.*, only phenylbutazone-induced ulceration was significantly effected. Atropine sulfate, used as a positive control in the phenylbutazone-induced ulceration experiment, significantly decreased the ulcer index at a dose of 10 mg/kg *i.p.* In the remaining three experiments, cimetidine (acting as a positive control) significantly decreased the

ulcer index. In the experiments employing aspirin and histamine, the decrease occurred at both 100 and 50 mg/kg *i.p.* In the stress-induced ulceration experiment, the decrease was effected only at a dose of 100 mg/kg *i.p.* (see Tables VII and VIII).

Effects of trans-2-Tridecenoic Acid on Contents of Mucous Components in the Glandular Portion of Rat Stomach

The hexosamine and sialic acid contents in the gastric mucous of phenylbutazone-induced ulcerated and intact rats treated with *trans*-2-tridecenoic acid (*i.p.*) was presented in Table IX. The administration of phenylbutazone (200 mg/kg *p.o.*) caused a marked decrease in hexosa-

TABLE V. *Effect of 2-Tridecenoic Acid (C_{13:1}) Administered Intraduodenally on Gastric Secretion in Pylorus-Ligated Rats (4 h)*

Treatment	Dose (mg/kg)	Gastric volume (ml/100g b.w.)	Total acid output (μeq/100g b.w.)	Total peptic activity (mg as tyrosine/100g b.w.)
Control ^{a)}	—	2.43±0.32	297.3±53.7	167.0±19.4
C _{13:1}	10	1.57±0.19*	164.0±18.0*	115.1± 9.6*
	5	1.91±0.19	223.1±35.7	138.1±16.7

All values represent mean ± s.e. (n=8).

a) 5% Acacia in saline.

Each sample was administered immediately after pylorus ligation (*i.p.*).

Significantly different from the control group: *p<0.05.

TABLE VI. *Effect of 2-Tridecenoic Acid on Gastric Ulceration in Pylorus-Ligated Rats (16 h)*

Treatment	Dose (mg/kg)	Ulcer index	Inhibition (%)
Control ^{a)}	—	3.5±0.3	—
C _{13:1}	10.0	2.1±0.3**	38.9
	5.0	2.7±0.3	22.6
	2.5	3.3±0.3	6.0
	—	4.2±0.3	—
Control ^{a)}	—	4.2±0.3	—
Cimetidine	100.0	2.9±0.4*	31.0
	50.0	3.7±0.4	11.9

All values represent mean ± s.e. (n=8).

a) 5% Acacia in saline.

Each sample was administered twice, immediately and 8 h after pylorus ligation (*i.p.*).

Significantly different from the control group: *p<0.05, **p<0.01.

mine and sialic acid levels in the gastric mucous and concomitant administration of *trans*-2-tridecenoic acid recovered hexosamine content and increased sialic acid content over the normal value (25 mg/kg *i.p.*). It was also found that the administration of *trans*-2-tridecenoic acid

TABLE VII. *Effect of 2-Tridecenoic Acid on Aspirin- or Phenylbutazone-Induced Ulceration in Rats*

Ulceration	Treatment	Dose (mg/kg)	Ulcer index
Aspirin-induced ulceration	Control ^{a)}	—	21.3±5.8
	C _{13:1} ^{b)}	25	7.0±1.5*
		10	11.5±3.2
	Control ^{a)}	—	20.2±4.7
	Cimetidine ^{b)}	100	3.2±1.1**
		50	6.4±1.0*
Phenylbutazone-induced ulceration	Control ^{a)}	—	13.5±3.5
	C _{13:1} ^{c)}	25	4.1±0.8*
		10	4.5±1.5*
	Atropine sulfate ^{c)}	10	2.6±0.5**

All values represent mean ± s.e. (n=8).

a) 5% Acacia in saline.

b) Each sample was administered immediately after pylorus ligation (*i.p.*).

c) Each sample was administered 30 min before the oral injection of phenylbutazone (*i.p.*).

Significantly different from the control group: **p* < 0.05, ***p* < 0.01.

TABLE VIII. *Effect of 2-Tridecenoic Acid on Histamine- or Stress-Induced Ulceration in Rats*

Ulceration	Treatment	Dose (mg/kg)	Ulcer index
Histamine-induced ulceration	Control ^{a)}	—	3.00±0.15
	C _{13:1} ^{b)}	25	2.13±0.35*
		10	2.88±0.23
	Cimetidine ^{b)}	100	1.14±0.26***
		50	2.00±0.22*
Stress-induced ulceration	Control ^{a)}	—	68.8±11.1
	C _{13:1} ^{c)}	25	26.0± 4.5**
		10	29.7± 6.6**
	Cimetidine ^{c)}	100	33.8± 4.5*
		50	54.6± 3.8

All values represent ± s.e. (n=8).

a) 5% Acacia in saline.

b) Each sample was administered 10 min before the injection of histamine (*i.p.*).

c) Each sample was administered twice, 15 min prior to stress exposure and 10 h later (*i.p.*).

Significantly different from the control group: **p* < 0.05, ***p* < 0.01, ****p* < 0.001.

TABLE IX. Effect of 2-Tridecenoic Acid on Hexosamine and Sialic Acid on the Glandular Portion of Phenylbutazone-Induced Ulceration in Rats

Treatment	Dose (mg/kg)	Hexosamine (μg glucosamine HCl/100 mg wet tissue)	Sialic acid (μg N-acetylneuraminic acid/100 mg wet tissue)
Control ^{a)}	—	312.0 $\pm$ 17.8	32.1 $\pm$ 1.4
Phenylbutazone (200 mg/kg)	—	206.8 $\pm$ 13.4	27.3 $\pm$ 3.0
Phenylbutazone (200 mg/kg) with C _{13:1} ^{b)}	25	355.4 $\pm$ 33.6**	38.7 $\pm$ 3.7*
Phenylbutazone (200 mg/kg) with C _{13:1} ^{b)}	10	206.4 $\pm$ 22.4	31.4 $\pm$ 2.3
Phenylbutazone (200 mg/kg) with atropine sulfate ^{b)}	10	570.6 $\pm$ 64.8***	54.5 $\pm$ 4.3***
Control ^{a)}	—	310.6 $\pm$ 18.6	26.1 $\pm$ 1.4
C _{13:1} single ad. ^{c)}	25	340.0 $\pm$ 25.9	34.1 $\pm$ 1.1***
C _{13:1} single ad. ^{c)}	10	314.1 $\pm$ 3.1	30.1 $\pm$ 2.2

All values represent mean $\pm$ s.e. (n=8).

a) 5% Acacia in saline.

b) Each sample was administered 30 min before the injection of phenylbutazone (i.p.).

c) Each sample was administered 5.5 h before rats were sacrificed (i.p.).

Significantly different from the phenylbutazone administered group (up) or control group (down):

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

FIG. 1. Effect of 2-Tridecenoic acid or Cimetidine on Gastric Secretion Stimulated by Histamine in the Perfused Stomach Preparation of Rats

↑ C_{13:1}:25 mg/kg i.p. or cimetidine: 100 mg/kg i.p.

● Histamine: 1.0 mg/kg i.v. (n=3).

TABLE X. *Effect of 2-Tridecenoic Acid on Gastric Emptying Rate in Rats*

Treatment	Dose (mg/kg)	No. of rats	Gastric emptying rate (%)
Control ^{a)}	—	4	59.4±4.9
C _{13:1}	25	4	50.2±2.7
	10	4	51.1±6.0

All values represent mean ± s.e.

a) 5% Acacia in saline.

Each sample was administered 40 min before the oral injection of phenol red (i.p.).

caused an increase in hexosamine and sialic acid contents of intact gastric mucous.

trans-2-Tridecenoic acid had no effect on the stimulated gastric juice secretion by tetragastrin or carbachol (not shown). However, the histamine enhanced gastric juice secretion in the stomach perfusion system was greatly reduced by the intraperitoneal injection of 25 mg/kg of *trans*-2-tridecenoic acid. The effect of cimetidine was also shown in the figure as a positive control (Fig. 1).

trans-2-Tridecenoic acid showed no effect on the gastric emptying rate at the dose of 25 and 10 mg/kg *i.p.*

DISCUSSION

In studying the structure-activity correlation of fatty acids, we examined the inhibitory activity of unsaturated fatty acids on gastric juice secretion in rats (*trans*-2-C_{10:1} to C_{16:1}, several C_{18:n} and C_{20:n}). *trans*-2-Tridecenoic acid (C_{13:1}) was found to be a potent inhibitor in this experiment. This result showed that, as was the result with saturated fatty acids, the length of the alkane chain of unsaturated fatty acids seemed to be an important factor for the inhibitory activity on gastric juice secretion. However, *trans*-2-tridecenoic acid (C_{13:1}) had about ten times the potential of myristic acid (C_{14:0}), which was a most potent inhibitor among even carbon number saturated fatty acids.

As a next step, we examined the effect of *trans*-2-tridecenoic acid on phenylbutazone-induced ulceration which is considered to cause

a destruction of the mucosal barrier and found a potent inhibitory effect. Kira *et al.*²⁰⁾ reported that among several gastric mucous components, hexosamine (as an indication of total mucopolysaccharide), sialic acid (glycoprotein) contents decreased when gastric ulcer was formed. Therefore, we examined the hexosamine and sialic acid content in gastric mucous of both intact and phenylbutazone administered rats after the intraperitoneal injection of *trans*-2-tridecenoic acid. *trans*-2-Tridecenoic acid increased the hexosamine and sialic acid content in both cases.

In ulcer formation mechanisms, two opposite factors are thought to be involved: an aggressive factor, such as gastric acid secretion, and a protective factor such as a mucosal barrier.²¹⁾ As shown in this article, *trans*-2-tridecenoic acid, was effective on both factors.

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