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Identification and Biological Activity of Fatty Acids as a Gastric Secretion Inhibitory Principle from Dried Brewer's Yeast[1]

Tsutomu Mimura, Hiroshi Tsujibo, Norio Muto, Satomi Otsuka, and Shigeru Aonuma

Faculty of Pharmaceutical Sciences, Osaka University[2]

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An active principle having gastric juice secretion-inhibiting activity in pylorus-ligated rats was purified from the ethanol extract of dried brewer's yeast by column chromatography, preparative thin-layer chromatography on silica gel, and gel filtration on a Sephadex LH-20. The active principle was a fatty acid mixture, consisting of $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{16:1}$ (palmitoleic acid), $C_{18:0}$, $C_{18:1}$ (oleic acid), and $C_{18:2}$ (linoleic acid) as determined by gas chromatography-mass spectrometry of the methyl esters. The biological activity of each identified fatty acid on gastric juice secretion was assayed in pylorus-ligated rats at a dose of 100 mg/kg (*i.p.*); the $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{15:0}$, and $C_{16:0}$ acids markedly decreased gastric juice volume, total acid output, and total peptic activity. Gastric volume and total acid output were significantly inhibited by $C_{16:1}$ acid, and the gastric volume was inhibited by $C_{18:2}$ acid. Among the $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{15:0}$, and $C_{16:0}$ acids, the $C_{12:0}$ and $C_{14:0}$ acids significantly prevented ulcer formation in pylorus-ligated rats.

Keywords——brewer's yeast; fatty acids; myristic acid; anti-ulcerogenic action; gastric juice secretion inhibitory activity

Since microorganisms produce such a wide variety of metabolites, it would seem reasonable to assume that they may include potentially useful pharmacologically active compounds. This work arose from attempts to find substances among microbial products that could inhibit experimental gastric secretion in rats.

We have been studying several microbial products with inhibitory action on gastric juice secretion; we have already purified an effective melanoprotein for peptic ulcers from *Streptomyces bottropensis*,[3] and we also obtained a gastric ulcer inhibitory glycoprotein as a metabolite of *Bacillus subtilis H*.[4]

The present work was undertaken to seek a new metabolite with inhibitory effect on gastric secretion and ulceration, using dried brewer's yeast as a material. We described here the purification of a fatty acid mixture as an active principle from dried yeast, and the identification and biological activities of each fatty acid component.

Materials and Methods

Dried yeast J.P. was kindly supplied by Ebios Yakuhin Co., Tokyo. Decanoic (purity, 98%), and dodecanoic (purity, 98%) acids were obtained from Katayama Chemical Co., Osaka, and tetradecanoic, pentadecanoic, 9-hexadecenoic, *cis*-9-octadecenoic, and *cis*-9, *cis*-12-octadecadienoic acids (purity, 99% each) from Wako Pure Chemical Industries, Ltd., Osaka. Hexadecanoic acid (purity, 99%) was obtained from Nakarai Chemicals, Ltd., Kyoto, and octadecanoic acid (purity, 100%) from E. Merck AG, Darmstadt, Germany.

Animals——Male Wistar rats weighing 150—200 g were used as experimental animals.

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2) Location: *133-1, Yamadakami, Suita-shi, Osaka-fu 565, Japan.*
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Extraction and Purification——One kilogram of dried brewer's yeast (*Saccharomyces cerevisiae*) was extracted three times with 3 liters each of hot EtOH, and the solvent was removed *in vacuo* to leave an oily residue. The residue was dissolved in $CHCl_3$ and, after the insoluble material had been removed, the solution was fractionated by silica gel column chromatography (Wakogel C-200). Stepwise elution of the column was carried out with the following solvent systems (v/v): (A) $CHCl_3$, (B) $CHCl_3$–Me_2CO (1:1), (C) Me_2CO, and (D) Me_2CO–EtOH (1:1). The fraction eluted with $CHCl_3$ showed inhibitory activity on gastric secretion in rats. This fraction (Fr. I) was concentrated under reduced pressure and the residue was purified by preparative thin-layer chromatography (TLC) (Wakogel B-5; solvent system, petroleum ether–Et_2O–AcOH=90:20:1 (v/v/v)), and the spot at *Rf* 0.46 showed the biological activity. This purified fraction (Fr. II) with inhibitory effect was further subjected to gel filtration on a Sephadex LH-20 column using EtOH as an eluant, and the active fraction (Fr. IIc) was purified. When Fr. IIc was further subjected to preparative TLC, a major component (*Rf* 0.46) and two minor components (*Rf* 0.21 and 0.08) were separated. These three spots were collected from the plates and the biological activity of each component was examined. The major component with the highest biological activity was designated as Fr. III. The total yield of Fr. III was about 150 mg from 1 kg of dried yeast.

Assay of Gastric Secretion Inhibitory Activity in Rats——The rats were deprived of food but allowed free access to water for 48 hr before the experiments. Under Et_2O anesthesia, the pylorus was ligated according to the method described by Shay *et al.*[5]

Each sample was ground to a fine powder with acacia (final concentration, 5%) and suspended in saline, and this suspension was administered intraperitoneally immediately after pylorus ligation. As a control, 5% acacia in saline was administered. After 4 hr, the animals were sacrificed and the stomach was removed. The gastric content was centrifuged and the volume of gastric juice was measured. Total acid output and total peptic activity were determined as described in our previous paper,[6] and they were expressed, respectively, as μEq/100 g body weight and mg as tyrosine/100 g body weight.

Anti-ulcerogenic Activity in Rats——Gastric Ulceration in Pylorus-ligated Rats: Male Wistar rats weighing 150 to 200 g, previously fasted for 24 hr, and pylorus-ligated as described above, were used. After 16 hr, the animals were sacrificed and the stomach was removed. The gastric mucosa was exposed by opening the stomach along the greater curvature and gastric ulcers that had developed in the forestomach were observed. The degree of gastric ulceration was estimated by the method of Narumi *et al.*[7] Ulceration was given an ulcer index from 0 to 5 according to its severity. Each fatty acid was intraperitoneally administered twice immediately and 8 hr after ligation.

Instrumental Analysis——Gas-liquid chromatography–mass spectrometry (GC–MS) was carried out with a Shimadzu LKB-9000 instrument equipped with a stainless steel column (0.3×200 cm), packed with 10% Silar 10C on Uniport KS (60—80 mesh). The column was operated at 170°.

Methylation of Fr. III——The active fraction (Fr. III) was methylated with CH_2N_2 and purified by thin-layer chromatography using the solvent system of petroleum ether–Et_2O–AcOH=90:20:1 (v/v/v). The methyl ester of Fr. III was detected with iodine vapor applied for just sufficient time to make it visible.

Results

Purification of the Active Principle

The gastric juice inhibitory principle was purified by extraction with ethanol, followed by column chromatography on silica gel and preparative TLC on silica gel (Fr. II). Since Fr. II thus obtained was not homogeneous, it was further subjected to gel filtration on a Sephadex LH-20, which gave three elution peaks as shown in Fig. 1. When Fr. IIc was subjected to preparative TLC again, the major component was separated as Fr. III. The effect of Fr. III on gastric secretion is shown in Table I.

Properties of Fr. III

Fr. III was soluble in most organic solvents, but insoluble in water; it was confirmed to be homogeneous by TLC and could be detected with iodine vapor. This fraction was presumed to be a mixture of fatty acids in view of the *Rf* value on TLC, and it was methylated with diazomethane for analysis by GC–MS.

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Identification of Fatty Acids Contained in Fr. III by GC-MS

As shown in Fig. 2, the methyl esters of Fr. III were separated into 9 components by gas-liquid chromatography, and the analytical result for each component obtained by mass spectrometry is shown in Table II. It was found that Fr. III was a fatty acid mixture, consis-

Table I. Inhibitory Effect of Fr. III on Gastric Secretion in Pylorus-ligated Rats (4 hr)

Treatment	Dose (mg/kg)	No. of rats	Gastric volume (ml/100 g b.w.)	Total acid output (μEg/100g b.w.)	Total peptic activity(mg as tyrosine/100 g b.w.)
Control[a)]	—	5	3.10±0.25	313.2±28.5	222.2±14.5
Fr. III	100	5	1.08±0.23[b)]	131.4±23.5[b)]	88.8±14.5[b)]

All values are means ± s.e.
a) 5% Acacia in saline.
Significantly different from the control group: *b*) $p<0.01$.

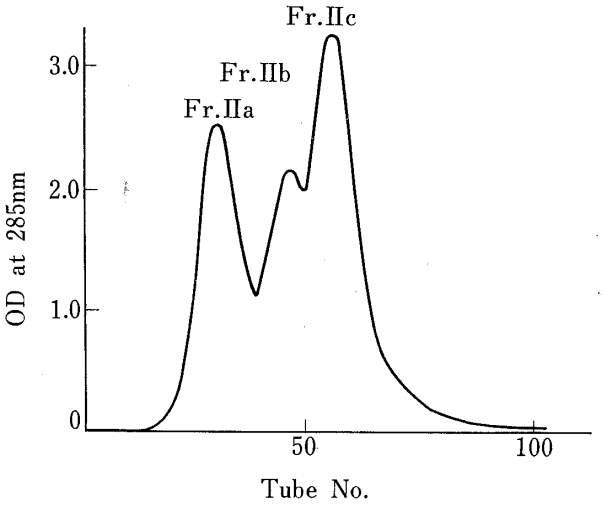

Fig. 1. Gel Filtration of Fr. II on a Sephadex LH-20 Column

Column size, 1.5×100 cm.
Solvent, EtOH.
Tube volume, 2.4 ml.

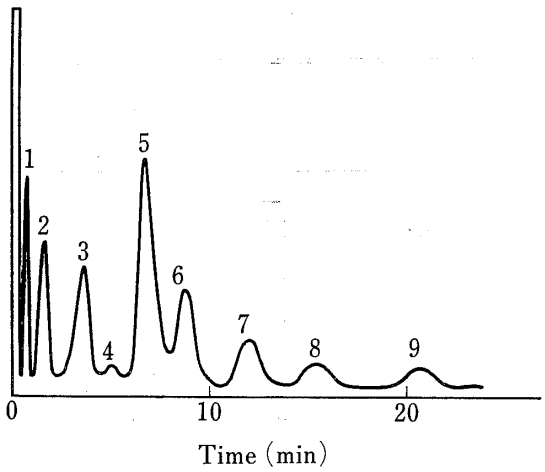

Fig. 2. Gas-Liquid Chromatogram of the Methyl Esters of Fr. III

Column size, 2 m×3 mm.
Column packing, 10% Silar 10C.
Column temp., 170°.
Injection temp., 270°.

Table II. Fatty Acids Present in Fr. III

Sample	Mol. wt.	Mass fragment	Structure	Fatty acid
1	186	74, 87, 143, M–43, M–31, M–29	$C_9H_{19}COOCH_3$	Capric acid
2	214	74, 87, 143, M–43, M–31, M–29	$C_{11}H_{23}COOCH_3$	Lauric acid
3	242	74, 87, 143, M–43, M–31, M–29	$C_{13}H_{27}COOCH_3$	Myristic acid
4	256	74, 87, 143, M–43, M–31, M–29	$C_{14}H_{29}COOCH_3$	Pentadecanoic acid
5	270	74, 87, 143, M–43, M–31, M–29	$C_{15}H_{31}COOCH_3$	Palmitic acid
6	268	55, 74, 87, M–116, M–74, M–32	$C_{15}H_{29}COOCH_3$	Palmitoleic acid
7	298	74, 87, 143, M–43, M–31, M–29	$C_{17}H_{35}COOCH_3$	Stearic acid
8	296	55, 74, 87, M–116, M–74, M–32	$C_{17}H_{33}COOCH_3$	Oleic acid
9	294	67, 81, 95, M–116, M–74, M–32	$C_{17}H_{31}COOCH_3$	Linoleic acid

ting of capric, lauric, myristric, pentadecanoic, palmitic, palmitoleic, stearic, oleic, and linoleic acids, based on the results of GC–MS analysis.

Gastric Juice Secretion Inhibitory Activity of the Identified Fatty Acids

The gastric juice secretion inhibitory activity of each fatty acid from the dried yeast is shown in Table III.

Table III. Effects of Various Fatty Acids on Gastric Secretion in Pylorus-ligated Rats (4 hr)

(A)

Treatment	Dose (mg/kg)	No. of rats	Gastric volume (ml/100 g b.w.)	Total acid output (μEg/100 g b.w.)	Total peptic activity (mg as tyrosine/100 g b.w.)
Control[a]	—	10	3.38±0.36	407.6±43.5	225.8±16.7
n-$C_{10:0}$	100	10	1.75±0.18[d]	176.4±19.5[d]	124.2±27.7[c]
n-$C_{12:0}$	100	10	1.79±0.23[c]	183.4±20.9[d]	115.2±16.9[d]
n-$C_{14:0}$	100	10	1.19±0.06[d]	114.1± 8.4[d]	106.1±15.2[d]
n-$C_{15:0}$	100	10	1.89±0.18[c]	216.0±28.7[d]	133.2±19.7[c]
n-$C_{16:0}$	100	10	1.78±0.22[c]	199.7±20.8[d]	110.6±13.2[d]

(B)

Treatment	Dose (mg/kg)	No. of rats	Gastric volume (ml/100 g b.w.)	Total acid output (μEg/100 g b.w.)	Total peptic activity (mg as tyrosine/100 g b.w.)
Control[a]	—	10	3.59±0.36	427.5±54.5	223.5±19.0
n-$C_{16:1}$	100	10	1.69±0.48[c]	155.1±50.6[c]	152.8±38.1
n-$C_{18:0}$	100	10	2.30±0.73	295.6±61.5	162.5±32.7
n-$C_{18:1}$	100	10	2.52±0.51	294.7±46.5	185.0±20.2
n-$C_{18:2}$	100	10	1.93±0.56[b]	298.8±56.5	185.3±32.5

All values are means ±s.e.
a) 5% Acacia in saline
Significantly different from the control group: *b*) $p<0.05$, *c*) $p<0.01$, *d*) $p<0.001$.

Intraperitoneal administration of capric, lauric, myristic, pentadecanoic, or palmitic acid (dose, 100 mg/kg) caused a significant reduction in gastric juice volume, total acid output, and total peptic activity. On the other hand, palmitoleic acid had an inhibitory effect on gastric juice volume and total acid output, and linoleic acid inhibited only gastric juice volume.

Table IV. Effects of Various Fatty Acids on Gastric Ulceration in Pylorus-ligated Rats (16 hr)

Treatment	Dose (mg/kg)	No. of rats	Ulcer index (mean±s.e.)
Control[a]	—	10	4.13±0.52
n-$C_{10:0}$	100×2[b]	10	3.58±0.63
n-$C_{12:0}$	100×2	10	1.67±0.58[c]
n-$C_{14:0}$	100×2	10	1.63±0.30[d]
n-$C_{15:0}$	100×2	10	3.00±0.67
n-$C_{16:0}$	100×2	10	3.20±0.61

All values are means±s.e.
a) 5% Acacia in saline.
b) Fatty acids were intraperitoneally administered twice, immediately and 8 hr after the ligation.
Significantly different from the control group: *c*) $p<0.01$, *d*) <0.001.

Anti-ulcerogenic Activity of Several Fatty Acids

The effects of capric, lauric, myristic, pentadecanoic, and plamitic acids, which decreased gastric secretion markedly, on ulceration in pyrlous-ligated rats are shown in Table IV. Lauric or myristic acid (100 mg/kg twice), when administered to pyrlous-ligated rats, significantly prevented gastric ulceration; the inhibition percentages in terms of the ulcer index were 59% and 60% respectively.

Discussion

To date only a limited number of substances among microbial products has been demonstrated to have an inhibitory effect on gastric secretion. Blickenstaff and Grossman[8] observed that gastric juice secretion was reduced in rats administered an exothermic factor, pyrexin, from *Pseudomonas aeruginosa*, while Taylor *et al.*,[9] Baume *et al.*,[10] and Wyllie *et al.*[11] obtained similar results with lipopolysaccharide from gram-negative bacilli. Based on these experiments, it seemed that the inhibitory effect of lipopolysaccharides depended on their toxicity as an endotoxin. Hence, it would be difficult to use them for medical treatment. On the other hand, it has been reported that peptide-mannan from baker's yeast inhibited gastric secretion.[12] The fatty acids obtained in the present work from brewer's yeast showed marked inhibitory activity.

Mammalian tissues contain large amounts of straight-chain fatty acids, but odd-numbered or branched-chain fatty acids have mainly been isolated from microorganisms.[13] In the present study, nine straight-chain fatty acids were isolated from dried brewer's yeast. Regarding the pharmacological activities of fatty acids, we have already reported an anti-inflammatory effect based on biomembrane stabilization by palmitic, isopalmitic, and isopentadecanoic acids.[14] Strong inhibitory activity on gastric secretion was found in even-numbered, saturated fatty acids (from $C_{10:0}$ to $C_{16:0}$), and the magnitude of the effects of these fatty acids seems to correlate well with the length of the hydrocarbon chain in each fatty acid. The structure–biological activity relationship of the fatty acids will be discussed in forthcoming papers.

We also examined the effects of five kinds of fatty acids, which markedly reduced gastric volume, total acid output, and total peptic activity, on ulceration in pyrlous-ligated rats. Lauric and myristic acids prevented ulcer formation in pylorus-ligated rats. It can be assumed that this effect is due to depression of gastric digestion due to the inhibition of gastric juice secretion by lauric or myristic acid. Further studies are required with various kinds of gastric ulcer models.

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