Arg, and Pro as amino acid moieties were observed on amino acid analysis. All of these results support the theory that the antibradykinin active material is a protein or a glycoprotein.

Action of the Antibradykinin Active Material Against Bradykinin Molecule—Bradykinin (0.5 mg) in 10 m*M* phosphate buffer (0.7 ml, pH 7.4) was incubated with 1.0 mg of Fraction D at 30° for 2 hr. The lyophilized mixture was subjected to paper electrophoresis with acetate–pyridine buffer (pH 3.5) to afford two ninhydrin positive spots of fast and slow moving ones compared with that of bradykinin which migrates towards the cathode. These two spots were not observed in the blank experiments without bradykinin or Fraction D.

The fast moving spot was eluted from unstained paper strips and the extract was subjected to amino acid analysis. The fast moving spot was confirmed to be a peptide composed of Arg, Pro, and Gly in the ratio of 1:2:1, which was consistent with the residues 1–4 (Arg^1-Pro^2-Pro^3-Gly^4) of bradykinin, indicating that Fraction D cleaved the bond between Gly^4 and Phe^5 in bradykinin and, therefore, had a kininase activity. The slow moving spot, however, was not clearly separated from the spot due to bradykinin on paper electrophoresis.

The incubation mixture after lyophilization was subjected to HPLC on a reversed-phase column. Three major peaks were observed at an elution volume of 6, 38, or 48 ml in HPLC, and each peak was collected and subjected to amino acid analysis to afford the following amino acid composition, respectively: Arg, Pro, Gly (1:2:1); Arg, Pro, Gly, Phe, Ser (1:3:1:1:1); and Arg, Pro, Gly, Phe, Ser (2:3:1:2:1).

Each composition was consistent with the structures Arg^1-Pro^2-Pro^3-Gly^4, Arg^1-Pro^2-Pro^3-Gly^4-Phe^5-Ser^6-Pro^7, and Arg^1-Pro^2-Pro^3-Gly^4-Phe^5-Ser^6-Pro^7-Phe^8-Arg^9 (bradykinin), respectively. All of these results indicate that the antibradykinin active material in *A. saponaria* has a kininase activity and cleaves the peptide bonds at *N*-terminuses of two phenylalanine residues in bradykinin.

As a kininase from plant origin, bromelain, papain, and ficin are known to cleave Gly^4-Phe^5 and Phe^5-Ser^6 bonds of the bradykinin molecule, while both shimejikininase (9) from mushroom, *Tricholoma congluobatum*, and kinonase AI (10) from microbes, *Streptomyces* species, cleave Gly^4-Phe^5 and Pro^7-Phe^8 bonds of the bradykinin molecule. Thus, the action of the glycoprotein obtained here is similar to that of shimejikininase and kinonase AI.

As one of the pharmacological evidences for anti-inflammatory activity of *A. saponaria*, the presence of an antibradykinin active glycoprotein was confirmed here. Further study on the anti-inflammation effect is in progress.

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Cardiotonic Principles of Ginger (*Zingiber officinale* Roscoe)

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Abstract □ Crude methanol extracts of the rhizome of ginger (*Zingiber officinale* Roscoe) showed potent, positive inotropic effects on the guinea pig isolated left atria. The extract of this rhizome has been fractionated, monitored by the cardiotonic activity, to yield gingerols as active principles.

Keyphrases □ Ginger (*Zingiber officinale* Roscoe)—cardiotonic principles, gingerols, □ Cardiotonic principles—ginger (*Zingiber officinale* Roscoe), gingerols □ Gingerols—cardiotonic principles of ginger (*Zingiber officinale* Roscoe)

The rhizome of ginger (*Zingiber officinale* Roscoe) has been used not only as a seasoning spice but also as an important medicine in Japan and China. It is considered to possess stomachic, carminative, stimulant, diuretic, bechic, and antiemetic properties (1). Chemical studies on the pungent principles of ginger have been carried out by a number of investigators (2–8). Recently, gingerols have been isolated from ginger as pungent substances (3).

It was found that the crude methanol extract of ginger had a powerful positive inotropic effect on the guinea pig isolated atria. The present report describes the isolation of cardiotonic principles from ginger and determination of their chemical structures.

EXPERIMENTAL[1]

Isolation—The dried rhizome (580 g) of ginger (*Z. officinale* Roscoe), zingiberis rhizoma, was crushed mechanically and soaked in methanol at room temperature. The mixture was filtered and the filtrate was evaporated *in vacuo*. The residue (106 g) was partitioned between water and ethyl acetate. The ethyl acetate layer was evaporated *in vacuo* and the residue (30 g) was dissolved in methanol and extracted with *n*-hexane. The pharmacologically active methanol fraction was again evaporated *in vacuo*. The residue (25.4 g) was dissolved in a small amount of benzene,

[1] Melting points were obtained on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotation was recorded on a Jasco ORD/UV-5 spectrometer with a circular dichroism attachment. UV spectra were obtained with a Hitachi 200-20 spectrophotometer. IR spectra were obtained on a Shimadzu LKB-9000B. PMR spectra were recorded on a Varian XL-100A spectrometer. CMR spectra were recorded on a Hitachi R-22 spectrometer.

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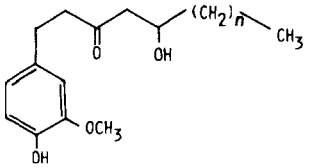

I : n=4 [6]-gingerol

II : n=6 [8]-gingerol

III: n=8 [10]-gingerol

put on a silica gel[2] column packed with benzene, and eluted with a mixture of benzene–ethyl acetate (9:1). Each fraction was monitored using TLC[3] with a mixture of benzene–acetonitrile (9:1) as a developing solution. Fractions showing the bioactivity were combined and rechromatographed five times on silica gel to afford three active substances, I–III:

Compound I—$C_{17}H_{26}O_4$, mp 30–32°, $[\alpha]_D$ = +27.8° (*C*, 1.0 chloroform); UV (ethanol): 284 nm (ϵ 2700); IR (neat): 1700 cm^{-1} (C=O); mass spectrum: *m/z* 294 (M^+, 61.8%), 137 (100), PMR (deuterochloroform): δ 0.88 (3H, broad t, J = 7 Hz, —$(CH_2)_4$—CH_3), 1.06–1.62 [8H, m, —$(CH_2)_4$—CH_3], 2.42–2.61 (2H, m, —CO—CH_2—CHOH—), 2.61–2.95 (4H, m, —CH_2—CH_2—CO—), 3.07 (1H, broad s, —*OH*), 3.84 (3H, s, —OCH_3), 3.86–4.16 (1H, m, —CH_2—*CH*OH—), 5.82 (1H, broad s, —*OH*), 6.54–6.86 (3H, m, aromatic H); CMR (deuterochloroform): δ 14.02 (q), 22.61 (t), 25.19 (t), 29.31 (t), 31.78 (t), 36.59 (t), 45.45 (t), 49.50 (t), 55.89 (q), 67.83 (d), 111.29 (d), 114.72 (d), 120.85 (d), 132.75 (s), 144.23 (s), 146.77 (s), 211.50 (s).

Compound II—$C_{19}H_{30}O_4$, mp 28–30°, $[\alpha]_D$ = +26.2° (*C*, 1.0 chloroform); UV (ethanol): 284 nm (ϵ 2700); IR (KBr): 1700 cm^{-1} (C=O); mass spectrum: *m/z* 322 (M^+, 13.9%), 137 (100); PMR (deuterochloroform): δ 0.88 [3H, broad t, J = 7 Hz, —$(CH_2)_6$—CH_3], 1.06–1.55 [12H, m, —$(CH_2)_6$—CH_3], 2.43–2.62 (2H, m, —CO—CH_2—CHOH—), 2.62–2.92 (4H, m, —CH_2—CH_2—CO—), 2.89 (1H, broad s, —*OH*), 3.86 (3H, s, —OCH_3), 3.88–4.16 (1H, m, —CH_2—*CH*OH—), 5.71 (1H, s, —*OH*), 6.56–6.88 (3H, m, aromatic H); CMR (deuterochloroform): δ 14.06 (q), 22.65 (t), 25.50 (t), 29.27 (t) × 2, 29.54 (t), 31.82 (t), 36.63 (t), 45.41 (t), 49.46 (t), 55.85 (q), 67.83 (d), 111.33 (d), 114.76 (d), 120.81 (d), 132.71 (s), 144.23 (s), 146.81 (s), 211.46 (s).

Compound III—$C_{21}H_{34}O_4$, mp 42–43°, $[\alpha]_D$ = +19.8 (*C*, 1.0 chloroform); UV (ethanol): 284 nm (ϵ 3100); IR (KBr): 1700 cm^{-1} (C=O); mass spectrum: *m/z* 350 (M^+, 13.4%), 137 (100); PMR (deuterochloroform): δ 0.80 (3H, broad t, J = 7 Hz, —$(CH_2)_8$—CH_3), 1.20 (16H, broad s, —$(CH_2)_8$—CH_3), 2.39–2.54 (2H, m, —CO—CH_2—CHOH—), 2.54–2.84 (4H, m, —CH_2—CH_2—), 2.91 (1H, broad s, —*OH*), 3.78 (3H, s, —OCH_3), 3.82–4.08 (1H, m, —CH_2—*CH*OH—), 5.63 (1H, broad s, —*OH*), 6.48–6.82 (3H, m, aromatic H); CMR (deuterochloroform): δ 14.09 (q), 22.69 (t), 25.50 (t), 29.35 (t) × 4, 29.62 (t), 31.93 (t), 36.63 (t), 45.45 (t), 49.46 (t), 55.89 (q), 67.83 (d), 111.25 (d), 114.68 (d), 120.85 (d), 132.75 (s), 144.19 (s), 146.73 (s), 211.50 (s).

Guinea Pig Isolated Left Atria—Bioassay of the fraction being tested was performed on the isolated left atria of guinea pigs. Guinea pigs (300–400 g) were sacrificed by cervical dislocation. The atrium was separated from the rest of the heart and mounted vertically in 50 ml of tissue bath containing Krebs-Ringer bicarbonate solution of the following

Rhizhome of ginger (580 g)
↓ extracted with methanol
Methanol extract (106 g)(+)
↓ partitioned with ethyl acetate and water

Ethyl acetate soluble portion (30 g)(+) — Water soluble portion (76 g)(−)
↓ partitioned with n-hexane and methanol

Methanol soluble portion (25.4 g)(+) — n-Hexane soluble portion (4.4 g)(−)
↓ chromatographed over silica gel
eluted with benzene-ethyl acetate (9:1)

(7.19 g)(−) | (7.29 g)(+) | (8.30 g)(−)
↓ chromatographed five times over silica gel
eluted with benzene-ethyl acetate (9:1)

Substance I (4.14 g)(+) | Substance II (0.62 g)(+) | Substance III (1.08 g)(+)

Scheme I—Procedure of the isolation of the cardiotonic principles from ginger (Z. officinale Roscoe); (+) active, (−) inactive.

[2] Silica gel 60, Merck.
[3] TLC plates silica gel 60 F_{254}, Merck.

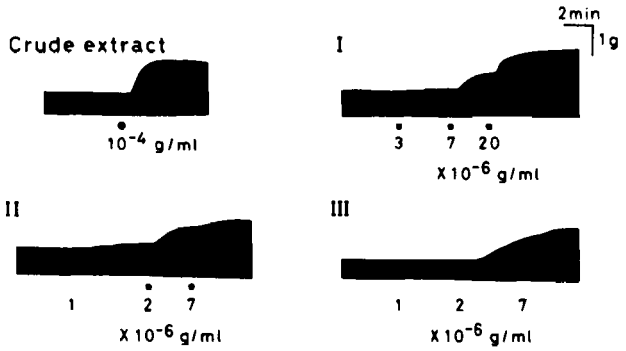

Figure 1—*The inotropic effect of the crude methanol extract of ginger (Z. officinale Roscoe) and gingerols on the guinea pig isolated left atria. Crude extract and gingerols were cumulatively added at (●). (I) [6]-gingerol; (II) [8]-gingerol; (III) [10]-gingerol.*

composition (in millimoles): sodium chloride, 120; potassium chloride, 4.8; calcium chloride, 1.2: magnesium sulfate, 1.3; potassium dihydrogenphosphate, 1.2; sodium hydrogen carbonate, 25.2; and glucose, 5.8; pH 7.4. The solution was bubbled with a gas mixture of oxygen–carbon dioxide (95:5) and maintained at 30°. A resting tension of 800 mg was applied to each strip. Tissues were driven by an electrical stimulator[4] at a frequency of 2 Hz with square-wave pulses of 5 msec at 4–5 V. Isometric contractions were measured by the force-displacement transducer and recorded on a polygraph. Four preparations from different animals were used for one sample being tested.

RESULTS AND DISCUSSION

The methanol extract (6×10^{-5}–3×10^{-4} g/ml) of rhizome of ginger caused a dose-dependent positive inotropic effect on the guinea pig isolated atria. A representative pattern of a positive inotropic effect of the methanol extract (10^{-4} g/ml) is shown in Fig. 1. In order to isolate the cardiotonic principles from ginger, fractionation of the methanol extract of ginger was performed, being monitored by the positive inotropic action as shown in Scheme I. Active fractions, 37–117, eluted with benzene–ethyl acetate (9:1) from the silica gel column, contained three components. The mixture was chromatographed five times over silica gel to give three active substances, I–III. Their yields were 0.71, 0.11, and 0.19%, respectively. Each substance tasted pungent, suggesting that they might be known pungent principles of ginger. Their physicochemical properties confirm that substances I–III are [6]-, [8]-, and [10]-gingerol, respectively, which had been isolated previously as pungent constituents (9).

As shown in Fig. 1, treatment with [6]-, [8]-, and [10]- gingerol of the atria induced a dose-dependent positive inotropic action. The minimum effective doses were 10^{-5}, 10^{-6}, and 3×10^{-5} g/ml for [6]-, [8]-, and [10]-gingerol, respectively. The activity appears to be in the decreasing order: [8]-gingerol > [10]-gingerol > [6]-gingerol.

On the basis of the present results, it is concluded that ginger has a powerful positive inotropic effect on the guinea pig isolated atria and that cardiotonic principles of ginger were identified as [6]-, [8]-, and [10]-gingerol.

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[4] Grass stimulator (model S9B).