Inhibitors of Platelet Aggregation Generated from Mixtures of *Allium* Species and/or *S*-Alk(en)nyl-L-cysteine Sulfoxides

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From the mixing of onion (*Allium cepa* L.) juice with *S*-alk(en)yl-L-cysteine sulfoxides or other *Allium* species, thiopropanal *S*-oxide, the lachrymatory factor of onion, reacted with other sulfenic acids produced by the action of CS-lyase (alliinase). Using this reaction, the AC series/cepaenes so far isolated from onion extracts as inhibitors of platelet aggregation and their homologues could be obtained in good yield. Hence, the mixing of onion with rakkyo (*A. chinense* G. Don) or garlic (*A. sativum* L.) resulted in marked increases in the inhibitory activity of platelet aggregation compared to the inhibitory activity of onion extracts. The antithrombotic compounds isolated from these mixtures were α-sulfinyl disulfides and thiosulfinates. Thiopropanal *S*-oxide was considered to play an important role in the formation of bioactive α-sulfinyl disulfides.

INTRODUCTION

It is well-known that *Allium* species produce a characteristic flavor when they are cut, crushed, or squeezed to juice. Their flavor is produced by the reaction of sulfenic acids derived from the action of CS-lyase (alliinase) with *S*-alk(en)yl-L-cysteine sulfoxides. Antithrombotic organosulfur compounds have been isolated and identified from *Allium* species. For example, methyl allyl trisulfide (Ariga et al., 1981), dithiin (Apitz-Castro et al., 1983), and ajoene (Block et al., 1984) were isolated from garlic, and AC series (Kawakishi and Morimitsu, 1988; Morimitsu and Kawakishi, 1990), cepaenes (Bayer et al., 1988), zwiebelanes (Bayer et al., 1989b), and butanedithial *S,S'*-dioxide (Block and Bayer, 1990) were isolated from onion. These compounds were considered to be generated from extremely reactive sulfenic acids just as the flavorous compounds of *Allium* species. Some of them possess other biological activities such as an antiasthmatic effect (Bayer et al., 1988, 1989a).

There are four *S*-alk(en)yl-L-cysteine sulfoxides (methyl, propyl, 1-propenyl, and allyl substituents) in *Allium* species. Some *Allium* species, garlic, onion, and rakkyo ("rakkyo" is the Japanese name for an *Allium* species similar to a scallion or shallot), contain predominant amounts of the specific *S*-alk(en)yl-L-cysteine sulfoxide with allyl, 1-propenyl, and methyl substituents, respectively. The inhibitory effects of platelet aggregation were individually tested in all *Allium* species, but no one has examined the inhibitory activities of their mixtures. In addition, *Allium* species that contain both *S*-allyl- and *S*-1-propenyl-L-cysteine sulfoxides as their constituents have not been reported. Some novel inhibitors of platelet aggregation may be found in the mixing extracts of onion and garlic.

The AC series were homologous to cepaenes in that they have an α-sulfinyl disulfide skeleton. These antithrombotic compounds were postulated to be produced by the addition of sulfenic acids to thiopropanal *S*-oxide (**1**), the lachrymatory factor (LF) of onion (Block et al., 1979). Yagami et al. (1980) have demonstrated the carbophilic addition of thiols to **1** (sulfine) by mixing the onion extract with benzenemethanethiol. The reaction products of **1** and methanesulfenic acid (or 2-propenesulfenic acid) can be observed in the mixture of onion and rakkyo (or garlic). Investigation of the antithrombotic compounds from these mixtures should help to clarify the formation pathway of the bioactive α-sulfinyl disulfides.

The present paper describes the isolation and identification of inhibitors of platelet aggregation from the extract of onion juice mixed with one of the *S*-alk(en)yl-L-cysteine sulfoxides (garlic or rakkyo). The proposed mechanisms for the formation of these compounds are also discussed with regard to the reaction of **1** and sulfenic acids.

EXPERIMENTAL PROCEDURES

Materials and Reagents. *Allium* species (onion, garlic, and rakkyo) were obtained from local markets in Nagoya, Japan. *S*-Methyl-L-cysteine was purchased from Sigma and oxidized to the sulfoxide according to the method of Synge and Wood (1956). Other *S*-alk(en)yl-L-cysteine sulfoxides were synthesized according to the methods of Freeman and Whenham (1975) and Lancaster and Kelly (1983). Disulfides were purchased from Wako Pure Chemical and Aldrich and oxidized to thiosulfinates with peracetic acid (Block and O'Connor, 1974; Block et al., 1986).

Equipment. Nuclear magnetic resonance (NMR) spectra were measured by a JEOL EX-270 spectrometer with tetramethylsilane (TMS) as internal standard. Electron impact mass spectra (EI-MS) were obtained using a JEOL DX-705L spectrometer. Infrared spectra (IR) were measured by a JASCO FT-IR 7000s spectrometer. The high-performance liquid chromatography (HPLC) system consisted of a pump (CCPE, TOSOH) and a normal-phase column (SI-60-5, 4.6 or 8.0 × 250 mm, Nomura Chemical) with a UV detector (UVIDEC-100-II, JASCO) at 270 nm.

Preparation of the Reaction Products from a Mixture of Onion Juice and *S*-Alk(en)yl-L-cysteine Sulfoxides or Other *Allium* Species. Onion bulbs (500 g) and a 10-mmol aqueous solution of *S*-alk(en)yl-L-cysteine sulfoxides (100 mL) or one of the other *Allium* species (garlic, 125 g; rakkyo, 500 g) were homogenized using a blender for 2 min at room temperature. After the homogenates were squeezed through nylon mesh (125 mesh) to afford onion juice, the solution was stirred for 2 h at room temperature. The reaction mixture was extracted with $CHCl_3$ (3 × 200 mL). The combined $CHCl_3$ extract was dried on anhydrous Na_2SO_4 and concentrated in vacuo at 20 °C, giving an oily residue.

Spectral Data of Antithrombotic Compounds Isolated from the Reaction Mixtures. No analytical data are presented for **3**, **4a,b**, **6a,7**, **7a,b**, **9a,b**, **10a,b**, **11a,b**, **16**, and **17**. 3-Ethyl-2,4,5-trithiahexane 2-*S*-oxide (**4a,b**), *trans*-3-ethyl-2,4,5-trithia-6-octene 2-*S*-oxide (**6a,b**), and *cis*-3-ethyl-2,4,5-trithia-6-octene 2-*S*-oxide (**7a,b**) were identical with AC-**1a,b**, AC-**11a,b**, and AC-

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12a,b isolated from onion as inhibitors of platelet aggregation (Morimitsu and Kawakishi, 1990), respectively. Methyl *cis*/*trans*-1-propenethiosulfinate (**3**), *trans*-5-ethyl-4,6,7-trithia-2-decene-4-S-oxide (**9a,b**), *trans,trans*-5-ethyl-4,6,7-trithia-2,8-decadiene-4-*S*-oxide (**10a,b**), and *trans,cis*-5-ethyl-4,6,7-trithia-2,8-decadiene 4-*S*-oxide (**11a,b**) were identical with cepaenes isolated from onion as dual inhibitors of cyclooxygenase and 5-lipoxygenase (Bayer et al., 1989a). *trans*-4,5,9-Trithiadodeca-1,6,11-triene-9-*S*-oxide (**16**) and *cis*-4,5,9-trithiadodeca-1,6,11-triene 9-*S*-oxide (**17**) were identical with (*E*)- and (*Z*)-ajoene isolated from garlic as antithrombotic compounds (Block et al., 1984), respectively.

***trans*-5-Ethyl-4,6,7-trithia-2-octene 4-*S*-Oxide (5).** EI-MS *m/z* (relative intensity) 210 (M^+, 1.5), 121 (92), 79 (42), 73 (100); ^1H NMR ($CDCl_3$) δ 1.16 (dd, 3 H, J = 7.2, 7.2 Hz, H-2′), 1.97 (dd, 3 H, J = 6.4, 1.1 Hz, H-8), 1.50 (ddq, 1 H, J = 14.8, 11.1, 7.2 Hz, H-1′), 2.29 (dqd, 1 H, J = 14.8, 7.2, 3.3 Hz, H-1′), 2.48 (s, 3 H, H-1), 3.52 (dd, 1 H, J = 11.3, 3.3 Hz, H-4), 6.44 (dq, 1 H, J = 15.0, 1.1 Hz, H-6), 6.55 (dq, 1 H, J = 15.0, 6.4 Hz, H-7).

5-Ethyl-4,6,7-trithiadecane 4-*S*-Oxide (12a). IR ν_{max} (CCl_4, cm^{-1}) 2950–2900 (s), 1220 (s), 1060 (s, sulfoxide); EI-MS *m/z* (relative intensity) 240 (M^+, 0.9), 149 (93), 117 (11), 107 (21), 73 (100); ^1H NMR ($CDCl_3$) δ 1.02 (dd, 3 H, J = 7.1, 7.1 Hz, H-2′), 1.14 (t, 3 H, J = 7.2 Hz, H-10), 1.22 (t, 3 H, J = 7.2 Hz, H-1), 1.68 (ddq, 1 H, J = 14.6, 11.3, 7.1 Hz, H-1′), 1.74 (tq, 2 H, J = 7.4, 7.2 Hz, H-9), 1.85 (tq, 2 H, J = 7.2, 7.2 Hz, H-2), 2.30 (dqd, 1 H, J = 14.6, 7.1, 3.7 Hz, H-1′), 2.65 (dt, 1 H, J = 12.2, 7.2 Hz, H-3), 2.77 (t, 2 H, J = 7.4 Hz, H-8), 2.78 (dt, 1 H, J = 12.2, 7.2 Hz, H-3), 3.66 (dd, 1 H, J = 11.3, 3.7 Hz, H-5).

12b (the Diastereoisomer of 12a). IR ν_{max} (CCl_4, cm^{-1}), 2950–2900 (s), 1220 (s), 1060 (s, sulfoxide); EI-MS *m/z* (relative intensity) 240 (M^+, 2), 149 (100), 117 (22), 107 (63), 73 (100); ^1H NMR ($CDCl_3$) δ 1.02 (dd, 3 H, J = 7.1, 7.1 Hz, H-2′), 1.12 (t, 3 H, J = 7.0 Hz, H-10), 1.16 (t, 3 H, J = 7.0 Hz, H-1), 1.72 (tq, 2 H, J = 7.3, 7.0 Hz, H-9), 1.91 (tq, 2 H, J = 7.8, 7.0 Hz, H-2), 1.96 (ddq, 1 H, J = 14.9, 11.1, 7.1 Hz, H-1′), 2.38 (dqd, 1 H, J = 14.9, 7.1, 3.3 Hz, H-1′), 2.71 (dt, 1 H, J = 12.9, 7.8 Hz, H-3), 2.74 (t, 2 H, J = 7.3 Hz, H-8), 3.12 (dt, 1 H, J = 12.9, 7.8 Hz, H-3), 3.48 (dd, 1 H, J = 11.1, 3.4 Hz, H-5); ^{13}C NMR ($CDCl_3$) δ 11.5 (C-2′), 13.3 (C-10), 13.7 (C-1), 16.9 (C-1′), 20.5 (C-9), 22.6 (C-2), 42.9 (C-8), 52.9 (C-3), 73.2 (C-5).

6-Ethyl-4,5,7-trithia-2-decene 7-*S*-Oxide (13; Cis,Trans Mixture). EI-MS *m/z* (relative intensity) 238 (M^+, 1.8), 179 (27), 147 (98), 105 (100), 73 (44).

***trans*-6-Ethyl-4,5,7-trithia-1,8-decadiene 7-*S*-Oxide (15).** EI-MS *m/z* (relative intensity) 236 (M^+, 0.8), 147 (100), 105 (69), 73 (71); ^1H NMR δ ($CDCl_3$) 0.92 (dd, 3 H, J = 7.3, 7.3 Hz, H-2′), 1.51 (ddq, 1 H, J = 13.8, 10.7, 7.3 Hz, H-1′), 1.78 (dd, 2 H, J = 7.8, 0.5 Hz, H-3), 1.97 (dd, 3 H, J = 6.5, 1.0 Hz, H-10), 2.32 (dqd, 1 H, J = 13.8, 7.3, 3.0 Hz, H-1′), 3.55 (dd, 1 H, J = 10.7, 3.0 Hz, H-6), 5.18–5.47 (m, 2 H, $CH_2CH{=}CH_2$, H-1), 5.87 (m, 1 H, $CH_2CH{=}CH_2$, H-2), 6.45 (dq, 1 H, J = 14.9, 1.0 Hz, H-8), 6.55 (dq, 1 H, J = 14.9, 6.5 Hz, H-9).

Enzyme Reaction of *S*-1-Propenyl- and *S*-Methyl-L-cysteine Sulfoxides. The preparation of S-substituted L-cysteine sulfoxide lyase (EC 4.4.1.4, CS-lyase; alliinase) from onion was based on that of Schwimmer and Mazelis (1963). Enzymic activity, as determined according to the method of Freeman and Whenham (1975), was 140 units/g of the crude enzyme containing CS-lyase. The crude enzyme (0.5 g) dissolved in 0.1 M Tris-HCl buffer (pH 8.5 at 37 °C, 15 mL), the buffer solutions of 0.25 mM pyridoxal phosphate (5 mL), 50 mM *S*-1-propenyl- and *S*-methyl-L-cysteine sulfoxide (5 mL each) were added all at once and incubated at 37 °C for 1 h with stirring. The reaction mixture was extracted with $CHCl_3$ (3 × 30 mL); the $CHCl_3$ phase was dried with anhydrous Na_2SO_4 and concentrated in vacuo. The reaction products were analyzed by HPLC [*n*-hexane/EtOAc/*i*-PrOH/EtOH 80/10/9/1 (v/v)].

Preparation of Human Platelets. Venous blood was obtained from healthy donors by drawing into plastic syringes containing 1/10 final volume of 3.8% sodium citrate as an anticoagulant. Platelet-rich plasma (PRP) was prepared by centrifugation of the citrated blood at 120*g* for 10 min. Platelet-poor plasma (PPP) was obtained from centrifugation of the residue at 1100*g* for 15 min. All procedures were performed at room temperature.

Table I. Inhibitory Effect of Mixtures of *Allium* Species on Human Platelet Aggregation

extract[a]	inhibition, % 20 μg[c]	10 μg[c]
onion	29	–[d]
rakkyo	0	–
garlic	100	57
onion plus rakkyo	79	11
onion plus garlic	100	100
garlic plus rakkyo	100	52

[a] The extraction was done by $CHCl_3$. [b] The inhibition percent was calculated from the decrease in maximal aggregation of the blank test induced by collagen. [c] This amount of each extract was added to 200 μL of PRP. [d] –, not measured.

Platelet Aggregation Measurement and IC_{50} Determination. Platelet aggregation was measured turbidimetrically using a dual-channel aggregometer (Hematracer I, NKK). PPP was used to adjust to 100% transmittance. For all experiments, 200 μL of PRP (250 000 platelets/μL) and 1 μL of the methanolic test sample solution were incubated at 37 °C for 1 min with stirring. The aggregation was induced by adding collagen (0.2 μg, fibrillar "Horm", Hormon-Chemie). This dose of collagen (1 μg/mL PRP) was determined to be the minimal required to elicit maximal platelet aggregation. Other inducers were often used with 20 μL of ADP solution (final concentration of 10 μM) or 20 μL of arachidonate solution (final concentration of 0.5 mM). The IC_{50} values were determined as the concentration of the compound that gives 50% inhibition of platelet aggregation compared with the blank test.

RESULTS AND DISCUSSION

Inhibitory Effect for Platelet Aggregation with Mixtures of *Allium* Species. Table I shows the inhibitory activities of the reaction mixtures. The mixtures of onion plus garlic and onion plus rakkyo exhibited a synergistic effect for inhibition of platelet aggregation compared to each individual one, but the mixture of garlic and rakkyo did not. The mixture of onion and *S*-allyl-L-cysteine sulfoxide instead of garlic also exhibited the synergistic effect, and the mixture of onion and *S*-methyl-L-cysteine sulfoxide instead of rakkyo similarly did (data not shown). These results suggested that the reaction products of 1-propenesulfenic acid and 2-propene- or methanesulfenic acid appear to possess the potent inhibitory activity.

Inhibitors Isolated from the Mixture of Onion and Rakkyo or *S*-Methyl-L-cysteine Sulfoxide (MCS). Compared with HPLC chromatograms of onion only, two chromatograms of the mixture of onion and *S*-methyl-L-cysteine sulfoxide and the mixture of onion and rakkyo (Figure 1) had obviously the same patterns (Figure 1B,C). Methanethiosulfinate (**2**) derived from methanesulfenic acids were detected in both mixtures. Inhibitors of platelet aggregation, methyl *cis*/*trans*-1-propenethiosulfinate (**3**), 3-ethyl-2,4,5-trithiahexane 2-*S*-oxide (**4a,b**), *trans*-5-ethyl-4,6,7-trithia-2-octene 4-*S*-oxide (**5**), *trans*-3-ethyl-2,4,5-trithia-6-octene 2-*S*-oxide (**6a,b**), and *cis*-3-ethyl-2,4,5-trithia-6-octene 2-*S*-oxide (**7a,b**) were isolated and identified. The yield of **5** was so small that its diastereoisomer could not be detected. All of them, except **5**, have already been isolated from onion only, while yields of **4a,b** and **6a,b** plus **7a,b** were dramatically increased over 40 and 30 times, respectively, as determined by the chromatointegrator connected to the HPLC (data not shown). Evidently, thiopropanal *S*-oxide (**1**) reacted with excess methanesulfenic acid, and all of its product contained methylsulfinyl and/or methane thioether functions. Another type of inhibitor could not be detected from these reaction mixtures.

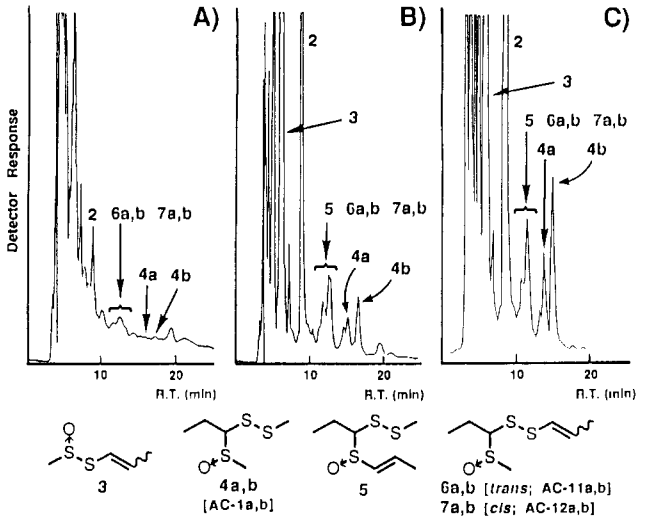


Figure 1. HPLC chromatograms of the extracts of onion (A), onion plus MCS (B), and onion plus rakkyo (C). Analytical conditions: flow solvent, EtOAc/$CHCl_3$/*i*-PrOH/EtOH 50/50/1/1 (v/v); flow rate, 0.8 mL/min.

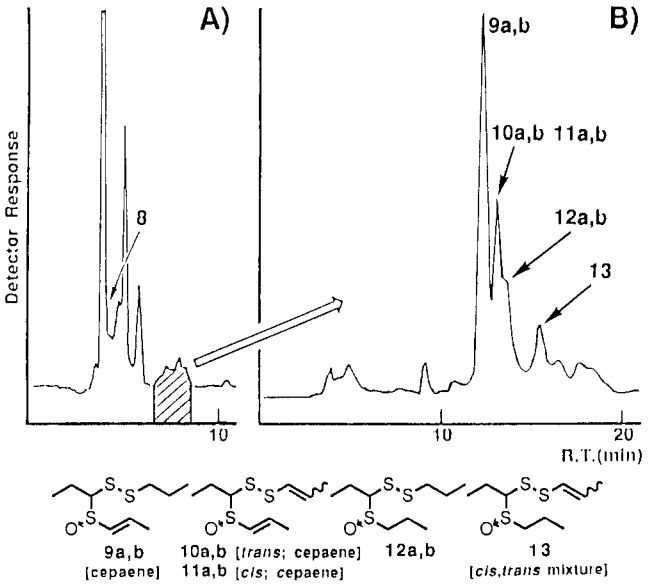


Figure 2. HPLC chromatograms of the extract of onion plus PCS (A) and the active fraction of this mixture (B). Analytical conditions: flow solvent of (A), *n*-hexane/EtOAc/*i*-PrOH/EtOH 80/10/9/1 (v/v); flow solvent of (B), *n*-hexane/EtOAc/EtOH 50/50/1 (v/v); flow rate, 0.8 mL/min.

Inhibitors Isolated from the Mixture of Onion and *S*-Propyl-L-cysteine Sulfoxide (PCS). *S*-Propyl-L-cysteine sulfoxide is one of the major sulfoxide derivatives in *Allium* species, especially in onion. An attempt to mix onion *S*-propyl-L-cysteine sulfoxide was carried out to determine how **1** could react with excess propanesulfenic acid just like methanesulfenic acid mentioned above. The HPLC chromatogram of this mixture is shown in Figure 2A. Propylthiosulfinate (**8**), derived from propanesulfenic acid, was detected in the mixture. Inhibitors of platelet aggregation, *trans*-5-ethyl-4,6,7-trithia-2-decene 4-*S*-oxide (**9a,b**), *trans,trans*-5-ethyl-4,6,7-trithia-2,8-decadiene 4-*S*-oxide (**10a,b**), *trans,cis*-5-ethyl-4,6,7-trithia-2,8-decadiene 4-*S*-oxide (**11a,b**), 5-ethyl-4,6,7-trithiadecane 4-*S*-oxide (**12a,b**), and 6-ethyl-4,5,7-trithia-2-decene 7-*S*-oxide (**13**; cis,trans mixture) were isolated and identified from the active fraction of the reaction mixture (Figure 2B). The new compound **13** was an analogue of the cepaenes, but its yield was too small to separate out each stereoisomer. The compounds **9a,b**, **12a,b**, and **13** were

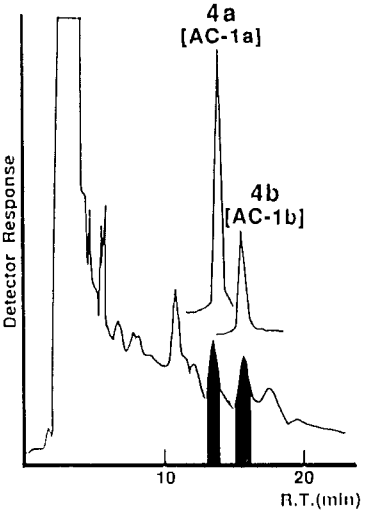
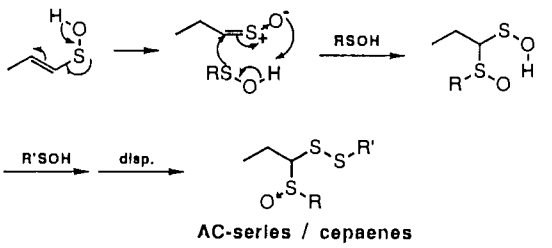


Figure 3. HPLC chromatogram of the extract of the model reaction of PCS plus MCS induced by CS-lyase. The analytical conditions were identical to those described in Figure 1.

Scheme I



produced by the reaction of **1** and excess propanesulfenic acid as expected. Also, another type of inhibitor could not be detected from this reaction mixture.

Model Reaction of *S*-1-Propenyl- and *S*-Methyl-L-cysteine Sulfoxides Induced by CS-Lyase. As additional model experiments of onion and rakkyo, the reaction of synthesized *S-cis*-1-propenyl- and *S*-methyl-1-L-cysteine sulfoxides with the crude enzyme prepared from onion was carried out. The reaction products extracted with $CHCl_3$ were directly analyzed by HPLC (Figure 3). Although the products **4a,b** could not be obtained in good yield, it was almost certain that they were formed by the reaction of 1-propenesulfenic acid and methanesulfenic acid. As a possible reason for their low yield, it was suggested that the precursor of the lachrymatory factor (LF, **1**) of onion was generated from the trans form of *S*-1-propenyl-L-cysteine sulfoxide but the synthesized compounds were almost always its cis form (cis/trans = 95/5 molar ratio determined by ^{13}C NMR). The principal component of the onion LF was determined to be the cis form of thiopropanal *S*-oxide (**1**) (Block et al., 1979). It is not clear how *cis*-1-propenesulfenic acid can be converted to *cis*- or *trans*-thiopropanal *S*-oxide and how the reactivity of *trans*-thiopropanal *S*-oxide with alkanesulfenic acid is different from that of the cis isomer.

Proposed Scheme for Formation of Inhibitors of Platelet Aggregation by Reaction of Thiopropanal *S*-Oxide and Alkanesulfenic Acids. From all of the results previously mentioned, we propose the formation scheme for inhibitors of platelet aggregation isolated from the mixture of onion and rakkyo or alkanesulfenic acids (Scheme I). By the action of CS-lyase, *trans*-1-propenesulfenic acid was produced and converted to the tautomer, thiopropanal *S*-oxide (**1**). The "carbophilic" mode of addition of 1-propene- or alkanesulfenic acid to **1** would generate a possible intermediate, α-[alk(en)ylsulfinyl]pro-

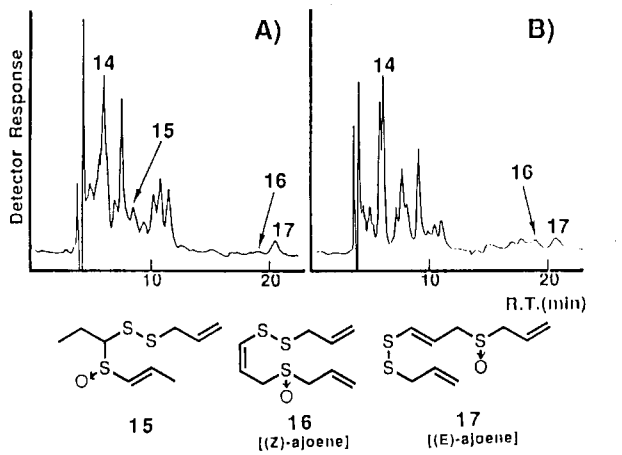


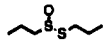
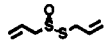
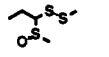
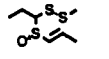
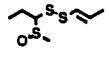
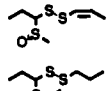
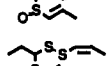
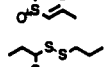
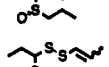
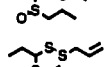
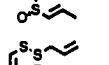
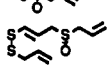
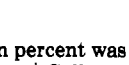
Figure 4. HPLC chromatograms of the extracts of onion plus ACS (A) and onion plus garlic (B). The analytical conditions were identical to those described in Figure 2A.

panesulfenic acid. The further reaction of this intermediate and another sulfenic acid would lead to inhibitors of platelet aggregation such as the AC series and cepaenes (Morimitsu and Kawakishi, 1990; Block and Bayer, 1990). Similarly, the carbophilic additions of a sulfenic acid to an olefin, sulfine, or thione were reported (Block and O'Connor, 1974; Yagami et al., 1980; Block and Aslam, 1985). It is worth noting that almost all of the potent inhibitors of platelet aggregation generated from **1** and alkanesulfenic acids were only α-sulfinyl disulfides. In other words, α-sulfinyl disulfide was the principal inhibitor derived from the reaction mixture of thiopropanal *S*-oxide and alkanesulfenic acid in onion.

Inhibitors Isolated from the Mixture of Onion and Garlic or *S*-Allyl-L-cysteine Sulfoxide (ACS). The inhibitory activity for platelet aggregation of the mixture of onion and garlic was significantly stronger than that of onion or garlic only (Table I). These data suggested that new inhibitors might be generated from this reaction mixture. HPLC chromatograms of both mixtures of onion and garlic or ACS were complicated and did not agree perfectly (Figure 4). The isolation of inhibitors was done from the mixture of onion and ACS (Figure 4A). Allylthiosulfinate (**14**) derived from 2-propenesulfenic acid was detected in the mixture. Inhibitors of platelet aggregation were isolated and identified as *trans*-6-ethyl-4,5,7-trithia-1,8-decadiene 7-*S*-oxide (**15**), *trans*-4,5,9-trithiadodeca-1,6,11-triene 9-*S*-oxide (**16**), and *cis*-4,5,9-trithiadodeca-1,6,11-triene 9-*S*-oxide (**17**). Compound **15** was analogous to cepaenes, but its yields were so small that its diastereoisomers could not be detected. Compounds **16** and **17** [(*E*/*Z*)-ajoene] were produced by the reaction of allylthiosulfinate and 2-propenesulfenic acid (Block et al., 1986). Total activities of these isolated compounds did not correspond to the increase in inhibitory activity for platelet aggregation of the mixture of onion and garlic. Only compound **15** was one of the reaction products of thiopropanol *S*-oxide and 2-propenesulfenic acid; moreover, **15** was one of the antithrombotic α-sulfinyl disulfides. Since our data on product analyses of onion and garlic are not sufficient to account for increasing inhibitory activity, further research on other inhibitors generated in the reaction mixture of thiopropanal *S*-oxide and 2-propenesulfenic acid will have to be done.

Antithrombotic Activity of Isolated Compounds. Isolated thiosulfinates, except methanethiosulfinate (**2**), possessed moderate activities: 10 μg each of **3**, **8**, and **14** inhibited platelet aggregation at about 41%, 20%, and 14% against blank tests, respectively. Other isolated α-sulfinyl disulfides and (*E*/*Z*)-ajoene (**16**, **17**) were tested for inhibitory ability of platelet aggregation (Table II). Some of them could not have IC_{50} values determined because of their small quantities. As shown in Table II, the AC series (**4a,b**, **6a,b**, **7a,b**), cepaenes (**9a**, **13a**), and (*Z*)-ajoene (**16**) exhibited potent activities. The novel homologues of the AC series and cepaenes, **12a,b**, indicated strong activity. Also, **13** and **15** showed mild activities. It is interesting that **5**, an isomer of **6a,b** (IC_{50} = 48.9, 11.7 μM), showed less activity compared to **6a,b**. The details of the relation between structure and activity are under consideration.

Table II. Inhibitory Effect of Isolated Compounds on Human Platelet Aggregation

compound No.	structure	inhibition(%)[a]	IC_{50} (μM)[b]
3		41.4	---[c]
8		20.0	---
14		14.3	---
4a,b		78.8 (4a) 100 (4b)	67.6 (4a) 18.4 (4b)
5		0.0	---
6a,b		92.8 (6a) 100 (6b)	48.9 (6a) 11.7 (6b)
7a,b		100 (7a) 100 (7b)	6.1 (7a) 1.4 (7b)
9a		77.1	---
13a		94.3	---
12a,b		100 (12a) 100 (12b)	5.1 (12a) 3.0 (12b)
13		17.1	---
15		21.4	---
16		90.7	---
17		14.3	---

[a] The inhibition percent was determined at 10 μg of each sample induced by collagen. [b] Collagen was used as inducer. [c] –, not determined.

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