assumption of lead poisoning as a pathogenic factor in ALS. They found that the CSF, blood, plasma and erythrocyte values of the metal did not differ appreciably from the controls (CSF Pb 0.89 ± 0.44 µg/l for the patients and 0.85 ± 0.91 µg/l for the controls). These results are in accordance with those of other investigators[7,8], who found no difference in CSF concentrations between patients and controls. Our results also could not confirm the findings of Conradi et al.[2] or the consideration of Cambell et al.[5]. The role of lead in producing the symptoms of ALS is as yet obscure, and the CSF levels of the metal found in this study do not indicate any correlation between lead and the disease, as concerns the CSF and serum Pb levels. Further investigations are required to elucidate this matter.

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Comparative platelet anti-aggregant activity of D-cysteinolic acid analogues

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Summary. D-Cysteinolic acid (**1**) analogues with an S-C-C-N skeleton showed increased platelet anti-aggregant activity in the following order: 2-aminoethanesulfonic acids, thiazolidines, 2-aminoethanethiols and 2-aminoethyl disulfides. Methyl substitutions at the 2-position potentiated the activity. Of these analogues, bis [(*R*)-2-aminopropyl] disulfide was the most potent inhibitor of platelet aggregation, with about 600-fold the activity of (**1**).
Key words. D-Cysteinolic acid; platelet aggregation; inhibitor; marine product; 2-aminoethyl disulfide; sardine.

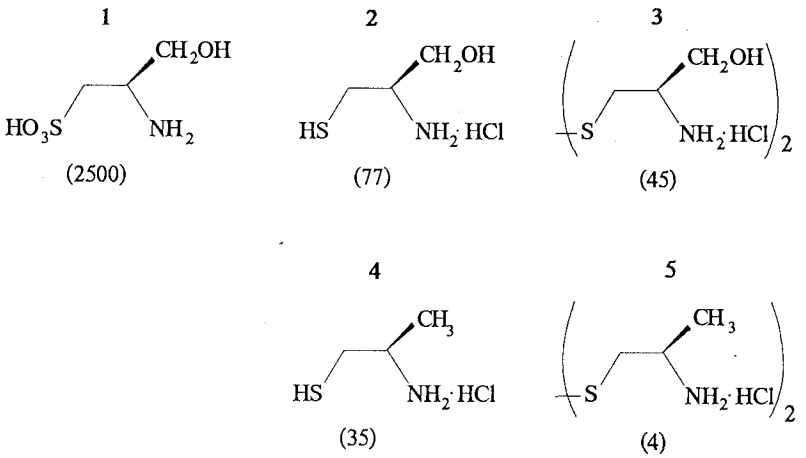
During our search for pharmacological activities of marine products we focused on D-cysteinolic acid (**1**), isolated from sardine which possesses platelet anti-aggregant activity, as it is a potentially good source from which to derive useful bioactive substances[1,2]. In the work described in this paper, in order to derive a more potent platelet inhibitor from **1**, comparative activities of analogues of **1** which have an S-C-C-N skeleton have been investigated.

Materials and methods

D-Cysteinolic acid prepared in our laboratory[2] was used in this experiment. Analogues **2**–**5**, listed in the figure, were synthesized according to standard procedure[3–8]. The details of the syntheses will be presented shortly. The structural proof of the analogue was based on proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy (fig.). Thiazolidine derivatives were synthesized as previously described[9]. The other analogues consisting of an S-C-C-N skeleton were purchased from Nacalai Tesque, Inc., Kyoto, Japan. Collagen (Type I), arachidonic acid (AA), platelet-activating factor (PAF), adenosine 5'-diphosphate 2Na (ADP), calcium ionophore (A-23 187), thrombin and epinephrine were obtained from Sigma Chemical Co., Mo, USA. Collagen (Horm) was provided by Niko Bioscience Ltd., Osaka, Japan, while all other reagents and solvents came from Nacalai Tesque. Platelet aggregation studies in vitro were performed according to the turbidometric method of Born and Cross[10] in an NKK Hema Tracer1 aggregometer. 25 test samples were added to 225 µl of platelet-rich plasma (PRP) 3 min prior to the addition of an aggregating agent. PRP was obtained from Wistar male rat, albino male rabbit or human blood[11]. Assay for AA-induced mortality in ddy male mice was carried out according to the method of Griffett et al.[12]. AA was administered 30 min after the injection of the sample, and mortality within 3 min after the AA injection was observed.

Results and discussion

Thiol (**2**) and disulfide (**3**) analogues of **1** were more potent inhibitors than **1** against collagen (Type III)-induced aggregation of rat PRP (fig.). Analogues **4** and **5**, with a methyl group were more potent than **2** and **3**, respectively, with a hydroxymethyl group. Of these analogues, **5** was the most potent inhibitor. The EC_{50} (estimated concentration to produce 50% inhibition) value was 4 µM for **5**. This analogue had about 600-fold the



Structure of D-cysteinolic acid analogues and their inhibitory activities in collagen (10 μg/ml)-induced rat platelet aggregation. **1**: D-cysteinolic acid, ^{1}H-NMR(δ,D_2O); 3.2(2H,m,-O_3SCH_2-), 3.6–4.0(3H,m, > $CHCH_2OH$); **2**: (*R*)-2-amino-3-mercaptopropanol, ^{1}H-NMR(δ,D_2O); 2.88(2H,m,$HSCH_2$-), 3.30(1H,m,-CH_2CH <), 3.80(2H,d,J = 5Hz,-CH_2OH); **3**: bis[(*R*)-2-amino-3-hydroxy-propyl] disulfide, ^{1}H-NMR(δ, MeOH-d_4); 1.42(4H,d,-CH_2OH ×2), 3.00–3.29(4H,m,-SCH_2- ×2), 3.40–3.71(2H,m,-SCH_2CH < ×2); **4**: 2-aminopropanethiol, ^{1}H-NMR-(δ,MeOH-d_4); 1.40(3H,d,J = 7Hz,CH_3-), 2.82(2H,m,$HSCH_2CH$ <), 3.40(1H,m,$HSCH_2CH$ <); **5**: bis[(*R*)-2-aminopropyl]disulfide, ^{1}H-NMR(δ,$CDCl_3$); 1.20(6H,d,J = 7Hz,CH_3- ×2), 1.59(4H, s,H_2N- ×2), 2.15(2H,dd,J = 25,12Hz,-SCH_2CH < ×2), 2.18(2H,dd,J = 23, 13Hz,-SCH_2CH < ×2), 3.07–3.44(2H,m,-SCH_2CH < ×2). ED_{50} values (μM) are given in parentheses.

activity of **1**. Aspirin, a standard platelet aggregation inhibitor, had an EC_{50} of 65 μM.

It is known that several sulfur-containing amino compounds from natural sources[9,13] (for example, taurine and cysteine) have weak activity against platelet aggregation. The EC_{50} values (μM) of **1** analogues with an S-C-C-N skeleton were as follows; cysteic acid, 2400; taurine, > 10 000; cysteine, 1100; cysteamine, 70; cystine, > 1000; cystamine, 23; (*R*)-thiazolidine-4-methanol, 140; (*S*)-thiazolidine-4-carboxylic acid, 6900 and thiazolidine, 900. These results gave regular structure-activity relationships. As a general rule, 2-aminoethane disulfides had the strongest activity, followed in order by 2-aminoethanethiols, thiazolidines and 2-aminoethanesulfonic acids. At the 2-position methyl substitutions increased the activity, but carboxyl ones decreased it. With the increase of hydrophobicity, platelet anti-aggregant activity seemed to become more potent. In any case, analogue **5** was the strongest inhibitor of collagen-induced aggregation of rat PRP. The EC_{50} values of **5** for several species and aggregating agents are shown in the table; the values for aspirin are included for comparison.

Platelet anti-aggregant activity of **5**

Aggregating agent	Concentration	Species	EC_{50} (μM) **5**	Aspirin
Collagen (Horm)	1 μg/ml	Human	75	160
(Type III)	10 μg/ml	Rabbit	4	100
ADP (2nd phase)	5 μM	Human	1	20
(1st phase)	16 μM	Rabbit	120	> 500
Epinephrine	10 μM	Human	60	100
Thrombin	0.5 U/ml	Rabbit	34	> 500
A-23 187	40 μM	Rabbit	400	[a]ND
PAF	0.1 μM	Rabbit	120	[a]ND
Arachidonate	0.5 mM	Rabbit	11	65
	[b]50 mg/kg, i.v.	Mouse	[c]12	[c]50

[a]Not determined; [b]Dose; [c]ED_{50}(mg/kg, i.p.).

Analogue **5** had strong activity (EC_{50}; 1 μM) against ADP (2nd phase)-induced aggregation of human PRP, but was less active against collagen (Horm) and epinephrine. In the aggregation of rabbit PRP, the inhibitory activities of **5** were in the order collagen (Type III) > AA > thrombin > ADP (1st phase), PAF > A-23 187. In collagen (Type III)-induced aggregations of rat and rabbit PRP, analogue **5** showed the same EC_{50} value (4 μM). Analogue **5** protected mice against AA-induced death. The estimated dose of **5** required to protect 50% of the animals from lethal effects of AA (EC_{50}) was 12 mg/kg i.p. Aspirin was considerably less effective than **5** in the present experiments.

It has been proposed that platelets are activated by various aggregating agents via receptor binding, phosphatidylinositol (PI) response, Ca^{2+} mobilization, phospholipase (PL) C activation and/or promotion of AA metabolism, followed by a release reaction (such as ADP release)[14–16]. On platelet activation, AA is physiologically converted to thromboxane A_2, which is a strong stimulator of platelet aggregation and release reaction, via prostaglandin endoperoxides, by various enzymatic reactions[17]. Analogue **5** had a greater effect against ADP (2nd phase) in human PRP, and collagen and AA in rabbit PRP, but was less active against ADP (1st phase), PAF and A-23 187 in rabbit PRP. Therefore, we consider that **5** affects AA metabolism rather than the other processes such as PI response, PLC activation and Ca^{2+} mobilization. Thus, analogue **5** was a potent inhibitor of platelet aggregation derived from the natural marine product.

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Splitting of the circadian activity rhythm in common marmosets (*Callithrix j. jacchus*; Primates)

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Summary. Long-term recordings of the free-running circadian activity rhythm in common marmosets, *Callithrix j. jacchus*, living under constant environmental conditions (LL 200–470 lx) provided evidence of rhythm splitting in nonhuman primates. In two out of ten test animals two different types of splitting occurred; spontaneous persistent splitting and temporary splitting. Neither a reduction of the illumination intensity nor the application of dark pulses had any effect on the two activity components of the persistently split rhythm.
Key words. Circadian rhythm; activity; splitting; Primates; *Callithrix*.

In the continuing discussion about whether one, two, or more, central nervous oscillators or pacemakers regulate the numerous circadian rhythms of the vertebrate organism, two phenomena are thought to point to the existence of a two- or multi-oscillator system; internal desynchronization and splitting. Spontaneous and/or forced internal desynchronization, in which various circadian functions display different period lengths for a long time, has only been observed thus far in man and in the diurnal squirrel monkey (*Saimiri sciureus*). Splitting has been found in several diurnal and nocturnal rodents, in tree shrews, and in certain species of birds, reptiles and fishes[1]. While free-running under constant environmental conditions, individual circadian rhythms dissociate (split) into two (or more) distinct components which either continually or only temporarily free-run with different spontaneous periods.

Splitting has been observed most frequently in locomotor activity. In a few cases, however, it has been reported that along with the splitting in the locomotor activity splitting may also occur in the free-running circadian rhythms of feeding, drinking and electrical brain self-stimulation[2] as well as in the serum concentration of luteinizing hormone[3]. Indications of splitting in the core temperature rhythm have only been observed in a few squirrel monkeys[4]. In these cases, however, the test animals had been restrained in a chair throughout the whole of a constant conditions experiment (LL 600 lx; 28 ± 1 °C) which lasted less than three weeks. Therefore it is not known whether splitting can occur in the activity rhythm as well, either in this diurnal Cebid species or in other nonhuman primates. We carried out two long-term constant conditions experiments to examine the possibility of social entrainment, and to test the effect of estrogen application on the circadian spontaneous period in free ranging common marmosets (*Callithrix j. jacchus*). During these experiments, we found individual cases of temporary and continuous splitting of the free-running circadian activity rhythm.

During the experiments, a total of 10 adult common marmosets (2 males and 8 females) aged 2–8 years were kept for 3–6 months in constant light (LL) of 200–470 lx at an ambient temperature of 25 ± 1 °C and 60 ± 5% relative humidity. The small diurnal monkeys lived individually housed in wire mesh cages (75 × 105 × 95 cm) which were placed in isolation boxes with thick (10 cm) sound-attenuating walls. The boxes were placed in the same room. Food, consisting of a protein-rich rice-flake porridge and mixed fruit, was provided ad libitum at irregular intervals once or twice per circadian cycle.

The locomotory activity of the test animals was recorded by a PC-controlled electroacoustic device. The vibrations (sounds in solids) of the wire mesh of the cage generated by the various activities of the animals (e.g., moving around, intense scratching, scent marking) were picked up by a transducer-like special microphone (Merula