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SHORT REPORTS

RELATION BETWEEN THE STRUCTURE OF ALLIIN ANALOGUES AND THEIR INHIBITORY EFFECT ON PLATELET AGGREGATION

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Key Word Index—Alliin; *S*-allyl-L-cysteine sulphoxide; allicin; dihydroalliin, platelet aggregation inhibitors

Abstract—Alliin analogues have been synthesized and tested for their inhibitory activity on platelet aggregation. It is found that only allicin, the *S*-oxodiallyl disulphide, has a strong inhibitory effect, comparable to that of alliin, while all the other tested compounds do not show any inhibitory effect even at concentrations of 10^{-3} M.

INTRODUCTION

The inhibitory effect of ethanolic and chloroform extracts of *Allium* sp. on human platelet aggregation has been reported [1, 2]. Most recently, Alliin (**1**), (+)-*S*-allyl-L-cysteine sulphoxide, was isolated from extracts of *Allium cepa* [3] and found to inhibit platelet aggregation. In an attempt to see if there is a structure–activity relationship, we examined several analogues of alliin, such as allicin and dihydroalliin, for their effect on platelet aggregation. It was found that only allicin (**2**), the *S*-oxodiallyl disulphide, similar to alliin, inhibits platelet aggregation.

RESULTS AND DISCUSSION

Alliin [4, 5], synthesized by us, was used as a reference compound to compare its action on platelet aggregation with all the other analogues examined. D- or L-Cysteine and L-methionine, as well as their sulphoxides, even at concentrations of 10^{-3} M, did not have any effect on platelet aggregation induced by either collagen or ADP (Table 1). *S*-Allyl-L-cysteine failed to inhibit platelet aggregation at a concentration of 1 mM.

S-Allyl-L-cysteine sulphoxide was found to have some inhibitory activity at a concentration of 10^{-3} M, whereas all the other analogues did not. *S*-Oxodiallyl disulphide, known as allicin, was found strongly to inhibit platelet aggregation induced by collagen (Fig. 1) or ADP (data not shown). Allicin is a well-known constituent of garlic with antibacterial properties [6, 7]. As already reported, aqueous solutions of allicin have a pH of *ca* 6.5 and, upon standing either at room temperature or 4°, an oily, yellow precipitate forms due to partial decomposition of allicin. Sulphur dioxide is formed during this period and the acidity of the solution increases [7]. It should be mentioned that the inhibitory effect of allicin in the precipitate form is less than that of freshly prepared alliin (Fig. 1). The inhibitory activity is completely lost when an aqueous solution of allicin is left at room temperature for more than 1 month due to complete decomposition of allicin.

Moreover, another sulphide, the methyl allyl trisulphide, has been isolated from garlic essential oils and also inhibits platelet aggregation [8]. We can, thus, conclude that the presence of an allyl group next to a sulphur atom is crucial for this biological effect. By replacement of the allyl group with propyl, trityl and benzyl groups, as in *S*-

Table 1 Effect of various alliin analogues on human platelet aggregation induced by collagen

Compound tested	IC_{50}
L-Methionine	—
D-Cysteine	—
L-Cysteine	—
L-Methionine sulphoxide	—
L-Cysteine sulphoxide	—
D-Cysteine sulphoxide	—
S-Allyl-L-cysteine	—
(±)-*S*-Allyl-L-cysteine sulphoxide	5.10
(+)-*S*-Allyl-L-cysteine (alliin)	0.06
S-Propyl-L-cysteine	—
(+)-*S*-Propyl-L-cysteine sulphoxide (dihydroalliin)	—
S-Oxodiallyl disulphide (allicin)	0.03
S-Benzyl-L-cysteine	—
S-Trityl-L-cysteine	—

The IC_{50} values are defined as the concentration of compound that gives 50% inhibition of platelet aggregation induced by collagen. (—) Even at the concentration of 1 mM no inhibition of platelet aggregation was observed

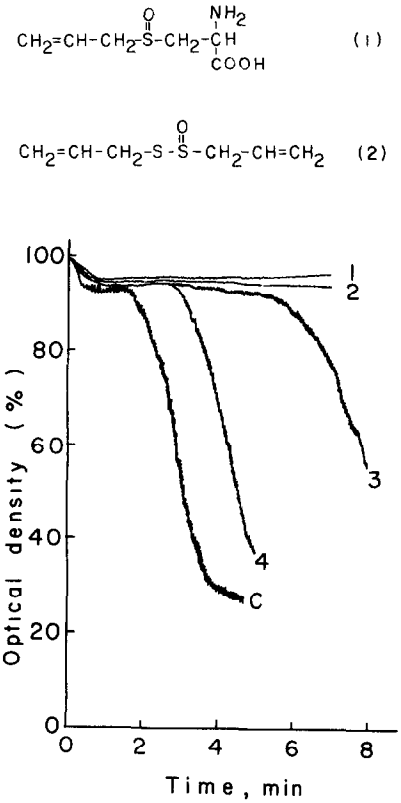

Fig. 1. Effect of alliin and allicin on platelet aggregation induced by collagen (1) Synthesized alliin (final concentration, 0.1 mM). (2) Freshly prepared allicin (final concentration, 0.05 mM). (3) Allicin in the precipitated form after 1 week (final concentration, 0 05 mM) (4) Allicin in the precipitated form after 1 month (final concentration, 0.05 mM). C, Control

propyl-L-cysteine, *S*-trityl-L-cysteine and *S*-benzyl-L-cysteine (Table 1), respectively, the inhibitory activity on platelet aggregation diminishes. Concerning the number of sulphur atoms present in the active compounds, it seems that a compound with two sulphur atoms (allicin) is more active than a compound with one (alliin). It will be interesting if a compound with three sulphur atoms, the methyl allyl trisulphide, is more effective than those with two sulphur atoms. Moreover, the active compounds alliin and allicin are also sulphoxides.

EXPERIMENTAL

Chemical syntheses. All sulphoxides were prepared by reaction of the sulphur-containing compound with 30% H_2O_2 at room temp for 30–45 min. They were ppted and recrystallized from Me_2CO.

S-Allyl-L-cysteine was prepared as follows: 2 g (8.3 mmol) L-cysteine was dissolved in 20 ml DMF and the pH adjusted to 8.2 with NEt_3 Allylbromide (1.2 ml) was then added drop-wise with continuous stirring The mixture was refluxed for 3 hr below the bp of allylbromide The reaction mixture was then evaporated to dryness, dissolved in a minimum vol. MeOH and then applied on a silica gel column (30 × 2 cm). The product was eluted with $CHCl_3$–MeOH (17:3) and recovered as fine crystals, mp 118–119° with a yield of 60% Its homogeneity was proved by TLC in $CHCl_3$–MeOH–HOAc (11:8.1) and *n*-BuOH–HOAc–H_2O (4:1·5).

(+)-*S*-Allyl-L-cysteine sulphoxide was prepared by reaction of *S*-allyl-L-cysteine with 30% H_2O_2 under the conditions mentioned previously. The separation of the two isomers was done as already reported [3, 7] by fractional crystallization. Both isomers were recovered as crystals of mp 162 and 150° and $[\alpha]_D^{20}$ + 59°, $[\alpha]_D^{20}$ − 58.1° (H_2O; *c* 2) for (+)- *S*-allyl-L-cysteine sulphoxide and (−)-*S*-allyl-L-cysteine sulphoxide, respectively.

S-Propyl-L-cysteine was prepared similarly to *S*-allyl-L-cysteine giving a yield of 65% and mp 120°.

Dihydroalliin was prepared from alliin by catalytic reduction with H_2/Ni as previously reported [6]. *S*-Oxodiallyl disulphide or 2-propene-1-sulphinethioic acid-*S*-2-propenylester [9] was prepared in two steps by reaction of allylbromide with NaSH followed by alkaline hydrolysis of CH_2=CH–CH_2–S–Na and by oxidation of the formed diallyl disulphide with H_2O_2 *S*-Oxodiallyl disulphide was obtained as a yellow liquid with a strong garlic-like flavour, bp 134° and yield of 43%. Its structure was further verified by IR and NMR.

S-Benzyl-L-cysteine and *S*-trityl-L-cysteine were purchased from Sigma All the other reagents used were of analytical grade Mps are uncorr.

Platelet aggregation inhibiting activity measurements Venous blood was obtained from healthy donors who had not taken any medication during the month preceding blood collection. The blood was immediately mixed in a 9·1 ratio with 3.8% sodium citrate and centrifuged at 200 *g* for 5 min to yield platelet-rich plasma.

Platelet aggregation was studied by a conventional photometric technique at 37° with continuous recording of light transmission (Dual Channel Coulter Electronic Aggregometer) according to ref [10]. The aggregating agents used were collagen (Sigma), at a final concn of 60 μg/ml and ADP (Sigma) at a final concn of 10 μM The inducer was added to the plasma rich in platelets (final vol 0.4 ml) after the addition of each compound examined

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