hygroscopic solid. $R_f = 0.30$ (system 3); UV λ_{max} nm: 282, 291. IR ν_{max} cm^{-1}: 3350, 2830, 2950. ^1H NMR (CD_3OD) (aromatic region): 7.25 (1H, *s*, H-2) 7.20 (1H, *dd*, $J = 8$ Hz, 8 Hz, H-6) 7.10, (1H, *dd*, $J = 8$ Hz, 2 Hz, H-7), 6.55 (1H, *dd*, $J = 8$ Hz, 2 Hz, H-5) MS *m/z*, 336 ($[M+1]^+$), 205, 160.

Enzymic breakdown of methoxyindole-3-methylglucosinolate. A soln of the natural methoxyindole-3-methylglucosinolate (10 mg) in buffer (pH 4) was incubated with myrosinase (2 mg) and ascorbic acid (2 mg) at 30° for 2 hr, centrifuged to remove proteinaceous material and the supernatant extracted (× 3) with EtOAc. The organic fractions were combined, dried (Na_2SO_4) and the solvent removed by evapn under red. pres. The residue was purified by prep. TLC (system 3) to afford a chromatographically pure sample of 4-methoxyascorbigen with identical MS, UV and ^1H NMR characteristics to the synthetic sample. Repetition of the above procedure followed by prep. TLC (system 2) gave a small yield of material with identical chromatographic, UV and MS characteristics to synthetic 4-methoxyindole-3-acetonitrile.

Enzymic breakdown of hydroxyindole-3-methylglucosinolate. A sample of the natural hydroxyindole-3-methylglucosinolate treated under similar conditions gave an unstable product with the ^1H NMR and MS characteristics of a hydroxyindole-3-acetonitrile. Immediate methylation by reaction with iodomethane and K_2CO_3 in Me_2CO [10] gave a product with identical chromatographic and spectral characteristics to authentic 4-methoxyindole-3-acetonitrile.

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IDENTIFICATION OF ALLIIN, A CONSTITUENT OF *ALLIUM CEPA* WITH AN INHIBITORY EFFECT ON PLATELET AGGREGATION

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Key Word Index—*Allium cepa*; Alliaceae; onion; platelet aggregation; alliin; *S*-allyl-L-cysteine sulfoxide.

Abstract—A component of *Allium cepa* which inhibits platelet aggregation *in vitro* was isolated. The active compound was identified as alliin, (+)-*S*-allyl-L-cysteine sulfoxide. Alliin was synthesized and found to exert the same activity on platelet aggregation as the natural compound.

INTRODUCTION

Baghurst *et al.* [1] first reported the effect of onions on platelet aggregation in people who had consumed a high fat diet. This observation was confirmed by others showing that chloroform extracts of the essential oils of *Allium cepa* and *A. sativum* inhibit platelet aggregation *in vitro*, induced either by ADP or by arachidonic acid [2, 3]. Philips and Poyser [4] have found that ethanol extracts of *Allium cepa* cause the same effect, while Ariga *et al.* [5] have isolated from garlic the methyl allyl trisulphide with inhibitory activity on platelet aggregation.

In this paper we describe the isolation and elucidation of the structure of the constituent of *Allium cepa* with inhibitory activity on platelet aggregation. The active

component was isolated by different chromatographic techniques and found to be (+)-*S*-allyl-L-cysteine sulfoxide, an analogue of cysteine known as alliin. Alliin was further synthesized in order to confirm the structure of the isolated substance and both the synthesized and isolated alliin exert the same effect on platelet aggregation.

RESULTS AND DISCUSSION

The purification of the active component was performed by different chromatographic techniques starting from 1 kg of onions. Homogenization of onions was done in a Waring blender for 5 min with 70% ethanol. The mixture was centrifuged at 8000 *g* and the ethanolic supernatant was evaporated to dryness. The powder was dissolved in distilled water and extracted with petrol and *n*-butanol, following the procedure of Phillips and Poyser [4]. The *n*-butanol-extracted material was chromatographed on a Sephadex LH-20 column (65 × 2.2 cm) equilibrated with methanol–water (1:1). The column was eluted with the same solvent and fractions of 7 ml were collected. Fractions with inhibitory activity on platelet aggregation were combined, concentrated by lyophilysis, and dialysed overnight against water. The dialysate, in which the activity was found, was lyophilized and the powder was dissolved in 2 ml of distilled water and applied on a Biogel P2 column. The active fractions (30–34) were combined, lyophilized and reapplied on the same Biogel P2 column. Again the active fractions (30–32) were combined, lyophilized and triturated with absolute methanol. The methanol soluble material was found to retain the activity against platelet aggregation. At this stage, enough material was collected and the active fractions were rechromatographed on a third Biogel P2 column.

Analysis of the purified material from the third Biogel P2 column by high-pressure liquid chromatography on a C18 column revealed the presence of seven components. Among these, tryptophan, phenylalanine, methionine, lysine, glutamine and asparagine were identified. None of these amino acids alone or in combination even at a concentration of 10^{-3} M was found to inhibit platelet aggregation *in vitro* induced by either collagen or ADP. The seventh component, which did not correspond to any of the common amino acids, was isolated in large quantities by preparative HPLC and was found to be very active. Thin-layer chromatography of the active component in the system *n*-BuOH–HOAc–H_2O (4:1:5) showed one ninhydrin-positive spot, R_f 0.52. The spot was also developed with iodine vapour. Finally, the active component was identified as (+)-*S*-allyl-L-cysteine sulfoxide, a known constituent of garlic named alliin [6]. *S*-Allyl-L-cysteine sulfoxide, which is racemic with respect to the sulfur atom, was first prepared following the procedure of Stoll and Seebeck [7]. From the mixture, alliin was obtained by fractional crystallization with acetone–water, as fine needles, mp 162–163°.

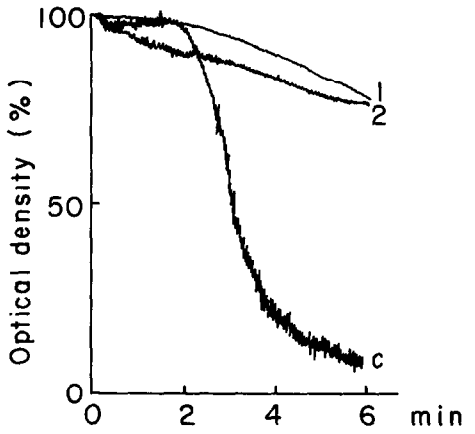

Fig. 1. Effect of allium on platelet aggregation induced by collagen. (1) Alliin isolated from *Allium cepa*; (2) (+)-*S*-allyl-L-cysteine sulfoxide (synthesized); (C) control.

Finally, the synthesized alliin was tested for its effect on platelet aggregation. Both the isolated and synthesized compound at a concentration of 0.1 mM inhibited platelet aggregation (*in vitro*) induced by collagen (Fig. 1).

EXPERIMENTAL

Plant material. Dried bulbs of common onions (*Allium cepa*) were used.

Chemicals. All the reagents and solvents used were of analytical grade. Sephadex LH-20 and Biogel P2 were purchased from Pharmacia and BioRad Lab, respectively

Bioassay. Platelet aggregation was studied by a conventional photometric technique, at 37°, with continuous recording of light transmission (Dual Channel Coulter Electronic Aggregometer). The aggregating agents used were collagen (Sigma) or ADP (Sigma) at concentrations 0.1 mg/ml and 0.5 mM, respectively. The inducer was added to the prp (plasma rich in platelets) medium after addition of plant material.

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