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SYNTHETIC *S*-ALK(EN)YL-L-CYSTEINE SULPHOXIDES–ALLIINASE FISSION PRODUCTS: SIMULATION OF FLAVOUR COMPONENTS OF *ALLIUM* SPECIES

GEORGE G. FREEMAN and ROBERT J. WHENHAM

Biochemistry Section, National Vegetable Research Station, Wellesbourne, Warwick CV35 9EF, England

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Key Word Index—*Allium* species; Liliaceae or Amaryllidaceae; onion; garlic; *A. aflatunense*; flavour simulation; *S*-1-propyl-, *S*-methyl-, *S*-2-propenyl- and *cis*-S-1-propenyl-L-cysteine sulphoxides.

Abstract—The preparation and properties (especially those of the respective alliinase-fission products) of synthetic flavour precursors as found in *Allium* species are described. Alliinase-fission products of mixtures of appropriate pairs of the precursors, after reduction with sodium borohydride, yielded volatile products which were substantially identical to the corresponding natural products.

INTRODUCTION

The preparation and properties of synthetic thiopropanal *S*-oxide and of a series of thiosulphinates and thiosulphonates involved as intermediates in the production of volatile flavour components of *Allium* species and their use in simulation experiments were described in an earlier paper [1]. This paper deals with simulation studies based on alliinase fission products of the corresponding synthetic *S*-alk(en)yl-L-cysteine sulphoxides.

RESULTS

Alliinase (EC 4.4.1.4, Alliin alkylsulphenate-lyase; alliin lyase). Alliinase was prepared by a method based on that of Schwimmer and Mazelis [2] as described by Freeman and Whenham [3]. Essentially the method consisted of extraction of fresh onion bulbs with buffer, precipitation of the enzyme with $(NH_4)_2SO_4$ and freeze-drying of an aqueous solution. The dry preparation had a specific activity of 100 units/g and contained about 17% of the activity of the starting material. The enzyme unit is defined as the amount of enzyme which will liberate 1 μmol of pyruvate/min under the specified conditions [3].

Mixture of (R) *and* (S)-*sulphoxides of* S-1-*propyl*-L-*cysteine* [(±)-S-1-*propyl*-L-*cysteine sulphoxide* (*PCSO*)]

The thioether (*S*-1-propyl-L-cysteine) was prepared by alkylation with 1-bromopropane of L-cysteine, formed *in situ* by reduction of L-cystine with Na in liquid NH_3 [4]. Oxidation with H_2O_2 gave the corresponding sulphoxide in 60% yield (Barnsley *et al.* [5]). Some properties of the sulphoxide have been described [3]. MS observations were consistent with theory for the sulphoxide. The compound ran as a single spot ($R_{cysteine}$ 1·23) on cellulose (Cel 400) TLC developed with 2-methylpropan-2-ol -formic acid (90%)–water (70 : 1 : 30, v/v) (solvent mixture I).

Enzymic fission. PCSO (6 μmol) in 0·1 M sodium pyrophosphate buffer pH 8·4 (3ml) was incubated at 37° for 30 min with an excess of the alliinase preparation (50 mg, 5 units) under the conditions described previously [3]. The reaction was arrested by addition of 10% trichloroacetic acid (7 ml) and the pyruvate content of the solution corresponded to 97% conversion.

A modified reaction mixture was used for observation of the UV spectrum of the products: PCSO (50 μmol) in 3ml of reaction mixture was incubated for 10 min with the same quantity of enzyme. The reaction was arrested by shaking with hexane (6ml). The UV spectrum of the hexane extract in the range 190–350 nm (λ_{max} 262–263 nm) was closely similar to that of 1-propyl propanethiosulphinate (λ_{max} 262 nm) [1]. The peak at 262 nm was eliminated to >95% completeness on shaking with L-cysteine reagent pH 4 [6]. Cellulose TLC on the aqueous layer from this procedure developed with 2-methylpropan-2-ol : formic acid (90%): conc-HCl: water (95 : 15 : 18 : 12, v/v) (solvent mixture II) [6] gave a single spot, $R_{cysteine}$ 2·09, in close agreement with the L-cysteine derivative of 1-propyl propanethiosulphinate $R_{cysteine}$ 2·07. By comparison of the A_{262} value of the hexane extract with that of a standard solution of the thiosulphinate, the content of the latter in the enzymic fission products was found to be 84% of theory and the pyruvate/thiosulphinate ratio was 2·4 molar.

Mixture of (R) *and* (S)-*sulphoxides of* S-*Methyl*-L-*cysteine* (*MCSO*)

MCSO was prepared by the above method. Overall yield 65%, mp 163° (cf. 163–165° [7]), IR: γ_{max}^{KBr} 1635, 2970 (amino-acid), 1020 (sulphoxide) cm^{-1}. Found: C, 31·9; H, 6·0; N, 9·2. Calc. for $C_4H_9O_3NS$: C, 31·8; H, 6·0; N, 9·3%. The sulphoxide ran as a single spot $R_{cysteine}$ 1·08 on TLC, developed with solvent mixture I. MS observations were consistent with theory for the sulphoxide.

Enzymic fission. Under the conditions described above, MCSO-alliinase mixtures gave > 95% conversion as pyruvate. Hexane extracts of the enzymic fission products

gave a shoulder at *ca* 260 nm, which was essentially eliminated on shaking with L-cysteine reagent pH 4. Because of the high partition coefficient of the thiosulphinate product, the UV spectrum in the range 190–350 nm was very similar to that obtained for a hexane solution of synthetic methyl methanethiosulphinate containing initially 0·28 μmol/ml, observed after aqueous extraction (cf. Fig. 2(d) [8]). TLC of the aqueous layer, after reaction with the L-cysteine reagent, using solvent II, gave a single spot, $R_{cysteine}$ 1·57, in agreement with the L-cysteine derivative of methyl methanethiosulphinate ($R_{cysteine}$ 1·45).

Mixture of (R) *and* (S)-*sulphoxides of* S-2-*propenyl*-L-*cysteine* (ACSO)

The corresponding 2-propenyl (allyl) sulphoxide was prepared by the same method. Overall yield of sulphoxide 60%, mp 163° (cf. 161–166° [9]), IR: γ_{max}^{KBr} 1600, 3000 (amino-acid), 1020 (sulphoxide), 920 (C=C) cm^{-1}, cf. natural alliin [10]. Found: C, 41·1; H, 6·3; N, 7·9. Calc. for $C_6H_{11}O_3NS$: C, 40·7; H, 6·2; N, 7·9%. The sulphoxide ran as a single spot $R_{cysteine}$ 1·68 on TLC (solvent I). MS data were consistent with theory.

Enzymic fission. Under the conditions described above, ACSO–alliinase mixtures gave >95% conversion as pyruvate. Hexane extracts of the fission products gave λ_{max} 262 nm, the peak being essentially eliminated (>95%) on reaction with L-cysteine reagent. TLC on the aqueous layer (solvent II) gave a single spot, $R_{cysteine}$ 2·02; standard based on synthetic 2-propenyl propenethiosulphinate, $R_{cysteine}$ 1·86.

Mixture of (R) *and* (S)-*sulphoxides of* cis-S-1-*propenyl*-L-*cysteine* (*PECSO*).

PECSO was not available by the above route but was prepared by base-catalysed isomerisation of *S*-2-propenyl-L-cysteine [4] with potassium *tert.*-butoxide in dimethyl sulphoxide to give *cis*-*S*-1-propenyl-L-cysteine in 55% yield [11]. The corresponding sulphoxide was obtained by H_2O_2 oxidation yield 60%, mp 140°d, cf. 138° [12]. IR: γ_{max}^{KBr} 1580, 2900 (amino-acid), 1020 (sulphoxide), 915 (C=C) cm^{-1}, cf. 950 cm^{-1}[10] in the natural *trans*-isomer. The sulphoxide ran as a single spot $R_{cysteine}$ 1·65 on TLC (solvent I). MS gave a complex fragmentation pattern consistent with the mechanisms postulated by Nishimura *et al.* [13] for flavour components of *Allium* species. Found: *m/e* 44 (100%), 45 (21%), 41 (19%), 39 (13%), 90 (9%), 73 (8%), 42 (7%), 74 (6%), 87 (5%), 43 (4%).

Enzymic fission. PECSO was relatively unstable, for example, under the slightly alkaline conditions (pH 8·4) of the standard enzymic assay mixtures, typical samples gave a maximum liberation of pyruvate of 79% [3]. Fission at pH 5·6 and 25° followed by hexane extraction gave λ_{max} 252 nm, compared with the corresponding extracts of fresh onion juice and of synthetic thiopropanal *S*-oxide, λ_{max} 254 nm. The peak obtained from PECSO was eliminated to 88% completeness on reaction with L-cysteine reagent pH 4 and on TLC (solvent II), the aqueous layer gave a single spot $R_{cysteine}$ 0·55 in agreement with a standard prepared from thiopropanal *S*-oxide, $R_{cysteine}$ 0·55. Based on PECSO (1·77 mg, 10 μmol) and 5 min reaction time, the pyruvate content of the enzymic-fission products was 2·82 μmol (28% conversion) and the thiopropanal *S*-oxide content was 1·28 μmol, giving a pyruvate/*S*-oxide ratio of 2·2 (theory 1·0). In a parallel experiment, eye tests showed the presence in the fission products of a powerful lachrymatory effect which was absent in the enzyme and precursor, tested separately.

Simulation experiments.

(a) *PECSO and MCSO.* Headspace vapours from mixtures of PECSO and MCSO in the molar proportions 10·9: 2·3 and of alliinase gave GLC-chromatograms practically devoid of significant peaks (relative total area of peaks emerging 6–60 min 3 cm^2) but addition of an excess of $NaBH_4$ (1 mmol) after 30 min incubation at 40° gave chromatograms (relative area 100 cm^2) essentially indistinguishable from that of fresh onion slices under comparable conditions. The major peaks (mono-, di- and tri-sulphides) are identified below, cf. also Fig. 1a [8]. A corresponding control experiment on the alliinase preparation + $NaBH_4$ gave small amounts of similar components (relative area 14 cm^2) owing to the presence of traces of flavour precursors which persisted in the alliinase preparation in spite of fractionation with $(NH_4)_2SO_4$.

(b) *PCSO and MCSO.* Corresponding mixtures of PCSO and MCSO (molar proportions 10·9: 2·3) except that the precursors were dissolved in the standard enzymic-fission mixture (3ml), pH 8·4 [3], gave similar results. The main difference between comparable chromatograms in the presence of $NaBH_4$ lay in the absence of a peak associated with the breakdown of thiopropanal *S*-oxide, namely 2-methylpent-2-enal, with PCSO as a component of the precursor mixture. The production of unsaturated (1-propenyl) as well as saturated disulphides under these conditions appears to be caused by dehydration of 1-propyl propanethiosulphinate [1].

(c) *MCSO.* Mixtures of MCSO (3·0 mg, 20 μmol), alliinase (50 mg, 5 units), buffer (3ml) and $NaBH_4$ (11 mg) under similar conditions gave methyl disulphide as the major component, with smaller amounts of methanethiol, methyl sulphide and methyl trisulphide. The chromatogram was closely similar to that produced by the headspace vapours from the sliced bulb of *A. aflatunense* B. Fedtschenko (6 g fr. wt), cf. Figure 1c [8].

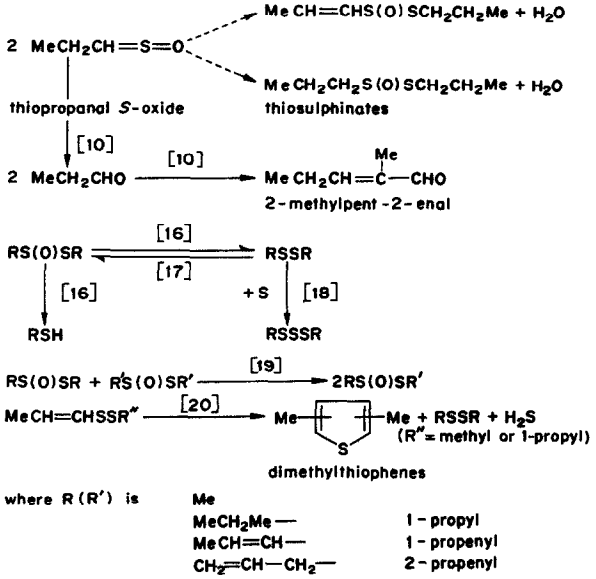
(d) *MCSO and ACSO.* Mixtures of MCSO (1·1 mg, 7 μmol), ACSO (7·7 mg, 43·5 μmol), alliinase (50 mg, 5 units), buffer (3ml) and $NaBH_4$ (11 mg) under similar conditions gave methyl-2-propenyl disulphide and 2-propenyl disulphide as the major components. Methyl disulphide, 2-propenyl trisulphide and minor components were also present. The chromatograms were essentially similar to those obtained from crushed garlic cloves (*A. sativum* L.) (18 g fr. wt) cf. Fig. 1b [8]. The chromatogram obtained from the precursors + enzyme had a total relative peak area of 1·7 cm^2, that from these components + $NaBH_4$, 100 cm^2 and the enzyme control (alliinase + $NaBH_4$), 5·7 cm^2. The characteristic peaks, containing 2-propenyl radicals, were absent from the enzyme control.

DISCUSSION

Evidence has been presented here that the alliinase-fission products of *Allium* flavour precursors such as PCSO, MCSO and ACSO contain the corresponding thiosulphinates as major components and those of PECSO the related thiopropanal *S*-oxide. The conclusion reached in an earlier paper [1] that these com-

pounds are important intermediates in the production of the characteristic volatile components, observed for example by GLC, is supported. The requirement for a suitable reducing agent, corresponding to the redox potential in the living plant, has also been demonstrated in the enzyme-precursor system.

Reactions by which the major compounds found by GLC may be formed from intermediate thiosulphinates and thiopropanal *S*-oxide are outlined below. Those based on the present work including [1] are shown with broken arrows.



EXPERIMENTAL

Details of the methods of GLC and GC–MS analysis and of the composition of the L-cysteine reagents have been given in an earlier paper [1]. Determination of pyruvate, thiopropanal *S*-oxide and thiosulphinates has been described [8].

Simulation experiment (*a*): *PECSO + MCSO*. A mixture of PECSO (19·3 mg, 109 μmol), MCSO (3·5 mg, 23 μmol) and alliinase (670 mg, 67 units) was dissolved in H_2O (10 ml). The proportions of the precursors were based on the quantities found by amino-acid analysis of onion by Matikkala and Virtanen [14], see also ref. [15]. The mixture was incubated at 40° for 30 min, followed by addition of $NaBH_4$ (38 mg, 1 mmol) and a further similar period of incubation. An aliquot (250ml) of the headspace vapours was collected and transferred to a GLC column as previously described [1]. The major peaks identified by retention data were equivalent to those found in fresh onion slices (10 g) under similar conditions and identified by GC–MS as follows: methanethiol, methyl sulphide, propanal, 1-propanethiol, methyl disulphide, 2-methylpent-2-enal, methyl-1-propyl disulphide, a mixture of methyl-*cis*-1-propenyl disulphide and 3,4-dimethylthiophene, 1-propyl disulphide, *cis*-1-propenyl-1-propyl disulphide, *trans*-1-propenyl-1-propyl disulphide and 1-propyl trisulphide, see also Fig. 1a [8]. The total GLC peak area (less enzyme and substrate blanks) for the simulation mixture was 89·5 cm^2, whereas fresh onion slices (10 g) gave 70 cm^2, under similar conditions. The relative proportions of 1-propyl:methyl:1-propenyl radicals in the volatile components from the synthetic precursors were 94 : 3·6 : 2·4 as compared with 88 : 6 : 6 reported for fresh onion [8].

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