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ENZYMATIC CONVERSION OF *TRANS*-(+)-*S*-1-PROPENYL-L-CYSTEINE *S*-OXIDE TO THE BITTER AND ODOR-BEARING COMPONENTS OF ONION

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Abstract—*Trans*-(+)-*S*-1-Propenyl-L-cysteine *S*-oxide, hitherto reported to be enzymatically transformed into the lachrymatory substance of freshly comminuted onion tissue, has now also been found to be the sole precursor of the substance responsible for the bitter taste in onion macerates. From a consideration of its concentration in onion, its properties as a substrate for enzyme action, and the relatively strong sensory responses elicited from such enzyme action, it is concluded this propenyl derivative is to a large degree responsible for the development of all of the sensory attributes perceived upon comminution of onion tissue.

INTRODUCTION

WHEN the structural integrity of onion tissue is destroyed by mastication or by other means of comminution, the sensory organs respond intensely in four major distinct ways, the sensations arising in the following order (Table 1). The eyes lachrymate; the tongue is

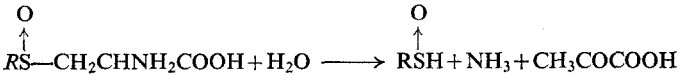
TABLE 1. ENZYMATIC DEVELOPMENT OF SENSORY ATTRIBUTES IN FRESHLY COMMINUTED ONIONS AND IN ENZYME REACTION MIXTURES CONTAINING HEATED ONION EXTRACT OR DERIVATIVES OF L-CYSTEINE *S*-OXIDE

Time, min	1	10	30	1	10	30	1	10	30	1	10	30
	Lachrymator			Bitterness			Astringency			Odor		
Substrate	Relative intensity of sensory attribute*											
Fresh onion	++	−	−	−	+	++	++	+	−	+	++	++
Heated onion	±	±	−	−	±	++	±	++	±	±	++	++
S-propenyl	±	±	−	−	±	++	++	+	±	±	++	++
S-allyl	−	−	−	−	−	−	±	++	±	++	++	++
S-propyl	−	−	−	−	−	−	+	++	±	+	++	++
S-methyl	−	−	−	−	−	−	−	±	−	±	+	+
Cycloalliin	−	−	−	−	−	−	−	−	−	−	−	−

* Intensity judged by the author, denoted as: ++, very strong; +, strong; ±, perceptible; −, absent.

subjected to a biting, burning astringency; the nose is accosted by the typical aroma of onion; and finally the comminuted tissue develops a distinct intensely bitter taste. As the result of investigations over the past 7 years, the biochemistry of the development of the odoriferous

and lachrymator components is fairly well understood in principle but not, as will subsequently be shown, in emphasis.[1] They arise from interaction of a pyridoxal enzyme with *S*-aliphatic-L-cysteine *S*-oxides



where *R* is methyl, propyl[2] and *trans*-propenyl.[3] (In garlic *R* is allyl.[4]) The unstable methyl and propyl sulfenic acids (RSOH) interact to form mixed volatile disulfide oxides (aliphatic thiosulfinates) which are considered to be responsible for the typical onion aroma. The lachrymator, the relatively stable propenyl sulfenic acid (half life 90 sec),[5] is converted at least in part to aldehydes and alcohols.[6] It has now been found that: (i) the substance(s) responsible for astringency arise from enzymatic action on (+)-propyl- and *trans*-(+)-propenyl-L-cysteine *S*-oxides (ii) the latter compound is the only precursor to the bitter principle in onion,[7] and (iii) the latter amino acid is preponderantly the principal progenitor of all four sensory attributes. The methyl and propyl derivatives play subsidiary roles.

EXPERIMENTAL AND RESULTS

The sequence of organoleptic changes of extracts from whole onion heated by microwaves to rapidly inactivate the naturally occurring enzyme, when incubated treated with enzyme present in a particulate fraction of onion homogenate, is qualitatively the same as (though quantitatively less intense than) those changes which occur upon comminution of fresh onion tissue (Table 1). These heated extracts thus contain the precursor(s) to both the astringent and bitter as well as the odor-bearing and lachrymatory principles. These observations thus demonstrate that all four sensory attributes arise as the result of enzyme action. The finding that the precursor to the bitter principle is retained by strong cation exchange resins is consonant with its being an amino acid.

Synthetic preparations (+)-*S*-methyl and (+)-*S*-propyl *S*-oxides[8] and a preparation of *trans*-(+)-*S*-propenyl-L-cysteine *S*-oxide isolated from onion were subjected to enzymatic action of the onion enzyme. As can be seen from Table 1, a reaction mixture containing either the propyl or propenyl derivative developed an onion-like odor and a burning sensation. However only the reaction mixture containing *S*-propenyl-L-cysteine *S*-oxide developed a discernible lachrymatory effect and was bitter. It was found that the most pronounced organoleptic characteristic of the reaction mixture containing *S*-methyl-L-cysteine *S*-oxide was a cooked-cabbage odor due presumably to dimethyl disulfide formed *via* dismutation of methyl methane thiosulfinate.[4] The reaction mixture containing the allyl derivative developed, as expected, a garlic odor[4] and was also strongly but transitorily astringent. Cycloalliin, a sulfur containing amino acid present in onion closely related to the propenyl congener,[9] did not develop any flavor.

The data of Table 1 thus strongly suggest that the precursor to the bitter principle in comminuted onion is, in fact, exclusively *trans*-(+)-*S*-propenyl-L-cysteine *S*-oxide. Further evidence is afforded by the procedure of isolation of this amino acid from onion by ion exchange chromatography using a modification of previous procedures.[3] Eluted fractions were assayed for the presence of this amino acid by adding enzyme and testing for bitterness. Only those fractions which gave a spot on paper chromatograms corresponding to that of an authentic sample, tasted bitter and were slightly lachrymatory after addition of enzyme.

[1] A. I. VIRTANEN, *Phytochem.* **4**, 207 (1965); S. SCHWIMMER and M. MAZELIS, *Arch. Biochem. Biophys.* **100**, 66 (1963); S. SCHWIMMER, *Biochem. Biophys. Acta* **81**, 377 (1964).

[2] A. I. VIRTANEN and E. J. MATIKKALA, *Acta Chem. Scand.* **13**, 1898 (1959); J. F. CARSON and F. F. WONG, *J. Org. Chem.* **26**, 4797 (1961).

[3] A. I. VIRTANEN and C. G. SPÅRE, *Suomen Kemistilehti* **B 34**, 72 (1961); J. F. CARSON, R. E. LUNDIN and T. E. LUKES, *J. Org. Chem.* **31**, 1634 (1966). The author thanks Professor A. I. Virtanen for an authentic sample of the propenyl derivative used as a standard in monitoring the isolation of this amino acid.

[4] A. STOLL and E. SEEBECK, *Advan. Enzymol.* **11**, 377 (1951).

[5] A. I. VIRTANEN and C. G. SPÅRE, *Acta Chem. Scand.* **17**, 641 (1965).

[6] C. G. SPÅRE and A. I. VIRTANEN, *Acta Chem. Scand.* **15**, 1280 (1961).

[7] S. SCHWIMMER, *Food Technol.* **21**, 292 (1967).

[8] A. STOLL and F. SEEBECK, *Helv. Chim. Acta* **34**, 481 (1951).

[9] A. I. VIRTANEN and E. MATIKKALA, *Acta Chem. Scand.* **13**, 623 (1959).

Further evidence of the identity of the precursor is afforded by the following properties of the product obtained *via* enzyme action on the *S*-propenyl derivative. Addition of L-cysteine to the bitter product (as a partially purified concentrate from fresh onion, or eluted from paper chromatograms of the enzyme reaction mixture) abolished the bitterness, intensified the odor, produced acid and a precipitate. These effects of cysteine are consonant with the presence of thiosulfinates and/or thiosulfonates, which are considered to be products of the action of *C-S* lyase on other cysteine *S*-oxide derivatives.[10]

A typical example of the course of production of pyruvic acid and bitterness from the propenyl derivative using the particulate fraction from onion as enzyme source is shown in Fig. 1. The reaction was conducted under conditions of enzyme concentration and pH comparable to those in a fresh onion homogenate. The concentrations of the methyl and propyl derivatives are based upon the data of Virtanen,[1] whereas that of the propenyl derivative is based on the yield obtained in the present investigation. This latter value, although higher than those reported in previous investigations,[3] still probably represents only a minor portion of the total present in onion since this amino acid is readily converted to cycloalliin under conditions of isolation.[1] The development of odor in the enzyme reaction mixture, like that of pyruvic acid production, was detectable at early stages of the reaction and increased with time. Close correlation between total pyruvic acid and odor

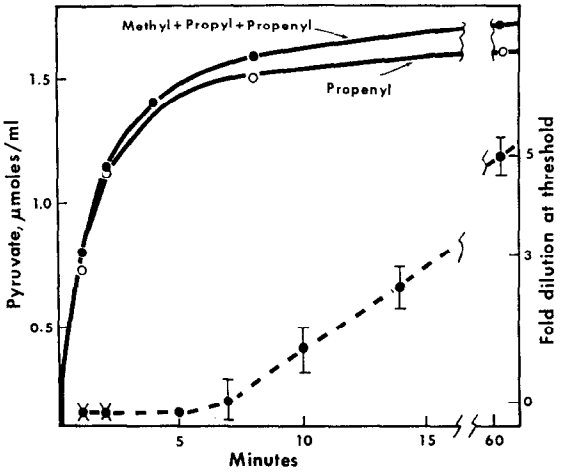


FIG. 1. COURSE OF PRODUCTION OF PYRUVIC ACID (SOLID CURVE) AND OF BITTERNESS (DASHED CURVE) IN THE PRESENCE OF *S*-PROPENYL-L-CYSTEINE *S*-OXIDE (2·0 μmoles/ml) AND IN THE PRESENCE OF THE SAME CONCENTRATION OF THE PROPENYL DERIVATIVE ACCOMPANIED BY THE METHYL DERIVATIVE (1·4 μmoles/ml) AND THE PROPYL DERIVATIVE (0·2 μmoles/ml).

Reaction run at 37° in the presence of 0·05 mg (equivalent to 0·5 g of tissue) of particulate fraction and 0·02 *M* potassium phosphate buffer pH 5·85. Pyruvic acid determined by the method of Schwimmer and Mazelis.[1] Bitterness expressed as magnitude of fold dilution of reaction mixture at taste threshold. The crosses indicate that no bitterness was detected at 0-fold dilution.

intensity has been reported.[11] By contrast, the bitterness was detected only after most of the substrate had been converted to pyruvic acid. This suggests that the development of bitterness is a secondary reaction with respect to pyruvic acid and odor formation, and that a second enzyme might be involved. However, similar results were obtained with a preparation of *C-S* lyase from *Albizzia lophanta* seed endosperm,[12] and also from the supernatant fraction of onion homogenate after removal therefrom of the bitter principle.

From a study of the effect of substrate concentration on the initial rate of onion *C-S* lyase action at pH 5·85, that of a typical onion homogenate, the relative calculated maximum initial rates of pyruvic acid formation, V_{max}, were in the proportion 1:1:3 for the methyl, propyl and propenyl derivatives, respectively (Table 2). The corresponding relative substrate concentrations at half maximal velocity (K_m) were in the proportion 6:2:1. From this data one can calculate that the initial rate of conversion of the propenyl derivative contributes at least 95 per cent of the initial rate in the presence of all three substrates in the proportion shown in

[10] D. BARNARD and E. R. COLE, *Anal. Chim. Acta* **20**, 540 (1959); L. D. SMALL, J. H. BAILEY and C. J. CAVALLITO, *J. Am. Chem. Soc.* **71**, 3565 (1949).

[11] S. SCHWIMMER and W. J. WESTON, *J. Agri. Food Chem.* **9**, 301 (1961); S. SCHWIMMER and D. GUADAGNI, *J. Food Sci.* **27**, 94 (1962).

[12] S. SCHWIMMER and A. KJAER, *Biochem. Biophys. Acta* **42**, 316 (1960).

Fig. 1. Even if all three substrates were present in equimolar quantities, the initial rate of conversion of the propenyl derivative would contribute five-sixths of the overall initial rate. During at least the first half of the reaction the course of pyruvic acid formation from such a multisubstrate mixture should, according to these calculations, be barely if at all experimentally distinguishable from that in the presence of propenyl derivative alone. The contribution of the other components should become experimentally apparent only at later stages of reaction. The data of Fig. 1 bear out these predictions. This supplemental action is probably responsible for the formation of mixed disulfides.[13] Wahlroos and Virtanen have given evidence of the presence of both *cis* and *trans* propenyl-propyl disulphide, and the probable occurrence of methyl-propenyl-disulphide, in the volatiles from the onion.[14]

TABLE 2. KINETIC PARAMETERS OF ONION *C-S* LYASE ACTING UNDER CONDITIONS COMPARABLE TO THOSE OF ONION HOMOGENATE

Substrate, *S*-(+)-cysteine *S*-oxide	Methyl	Propyl	Propenyl
Substrate, μmoles/g onion	1·3	0·2	2·0
Substrate, contribution to total, %	37	6	57
K_m, μmoles substrate/ml	34	11	6
V_{max}, μmoles pyruvate/ml/5 min	0·9	0·9	2·9
Contribution to rate (separately), %	4·5	2·0	93·5
Contribution to rate (together), %	3·3	1·6	95·1

One ml of enzyme reaction mixture contained: substrate, μmoles as shown in table; enzyme particulate fraction corresponding to 0·5 g of onion; potassium phosphate buffer pH 5·85; 40 μmoles; pyridoxal phosphate, 0·05 μmoles.

The intensity of onion odor of individual enzyme reaction mixtures each containing the propenyl propyl or methyl derivative of L-cysteine *S*-oxide as substrate and a sample containing enzyme but no substrate was appraised by a panel of 16 trained judges. Statistical analysis of the results showed that the reaction mixture containing the propenyl substrate had a significantly more intense onion odor ($P < 0{\cdot}01$) than did any of the other reaction mixtures.

Thus from consideration of yield, enzyme kinetics, and intensity of sensory response during enzyme action, it would appear that *trans*-(+)-*S*-propenyl-L-cysteine *S*-oxide is preponderantly the most significant progenitor of the sensory properties of onions. An outline of the major conversion of the *S*-substituted cysteine *S*-oxides in onion and the relation to the sensory properties of onions is shown in Table 3. It is obvious that much remains to be learned of the nature of the complex mixture comprising the flavor constituents of onion.

TABLE 3. PROPOSED SCHEME FOR DEVELOPMENT OF THE SUBSTANCES RESPONSIBLE FOR THE MAJOR SENSORY ATTRIBUTES OF ONION

Compounds	Organoleptic attribute	*R*
R—SO—CH_2—CHNH_2—COOH		
(L-cysteine *S*-oxide)	None	I, II, III
R—SOH	Lachrymatory effect	I
(sulfenic acid)	Biting astringency	II, III
R—*S*—SO—*R*		
(thiosulfinate)	Odor	I, II, III
R—*S*—*S*—*R* + *R*—*S*—SO_2—*R*		
(disulfide + thiosulfonate)	Odor	I, II, III
X—*S*—SO_2	Bitterness	I

The substances are listed in order of appearance starting with the L-cysteine *S*-oxide progenitors. *R* denotes the propenyl (I), propyl (II), and methyl (III) derivatives. *X*—*S*—SO_2 denotes unknown thiosulfonate derivative(s).

[13] J. F. CARSON and F. F. WONG, *J. Agri. Food Chem.* **9** (2), 140 (1961).
[14] Ö. WAHLROOS and A. I. VIRTANEN, *Acta Chem. Scand.* **19**, 1327 (1965).