supplied giving an unknown actual concentration in the aqueous phase because of the wide variation in water solubility and volatility of the thiols used. After 30 min acetic acid was added to stop the reaction, and the mixture was dried in a vacuum desiccator. The reaction products were dissolved in water and subjected to thin layer chromatography with methyl ethyl ketone/pyridine/water/acetic acid (70 + 15 + 15 + 2 v/v) followed by autoradiography.[1,5]

Results. When the enzymes from onion and *E. coli* were incubated with labeled *O*-acetylserine and one of the thiols methyl, ethyl, propyl, allyl, or benzyl mercaptan, distinct spots of *S*-methylcysteine, *S*-ethylcysteine, *S*-propylcysteine, *S*-allylcysteine, and *S*-benzylcysteine, respectively, appeared on the autoradiograms. When butyl mercaptan was tested the reaction was only weak, which may reflect the low water solubility of this compound.

Discussion. Although the biosynthesis of *trans*-(+)-*S*-(propen-1-yl)cysteine sulfoxide from *S*-(2-carboxypropyl)cysteine in onion has been elucidated the origin of the side chain in other *S*-substituted cysteine derivatives has remained unknown.[1] At present, there is no evidence about the origin of the allyl side chain in *S*-allylcysteine sulfoxide in garlic. The side chain of *S*-propylcysteine sulfoxide, a minor component in onion, may be formed by hydrogenation of propenylcysteine sulfoxide, although this has not yet been experimentally proved. The metabolism of *S*-methylcysteine and its sulfoxide has been studied in several higher plants (for literature, see the reviews of Thompson [6] and Granroth [1] and some more recent publications [7-11]). This compound can be formed either by methylation of cysteine or by thiomethylation of serine, but the former reaction seems to be the normal one.

The demonstration in this investigation of the non-specific action of cysteine synthase from two different sources suggests that the ability to form cysteine derivatives from thiols and serine is wide-spread and may be an important detoxification mechanism. In onion sulfur metabolism it explains the side-chain recycling mechanism previously observed.[1] This is a reaction in which the thioalkyl moiety of an *S*-substituted cysteine derivative is combined with serine to form a new molecule with the same structure as the original. This reaction is sometimes confusing and makes the investigation of onion sulfur metabolism more intricate.

1. Granroth, B. *Ann. Acad. Sci. Fenn. Ser. A 2*, No. 154 (1970).
2. Granroth, B. *Acta Chem. Scand. B 28* (1974) 813.
3. Frankel, M., Cordova, S. and Breuer, M. *J. Chem. Soc.* (1953) 1991.
4. Becker, M. A., Kredich, N. M. and Tomkins, G. M. *J. Biol. Chem. 244* (1969) 2418.
5. Granroth, B. *Acta Chem. Scand. 22* (1968) 3333.
6. Thompson, J. F. *Annu. Rev. Plant. Physiol. 18* (1967) 59.
7. Chen, D. M., Nigam, S. N. and McConnell, W. B. *Can. J. Biochem. 48* (1970) 1278.
8. Doney, R. C. and Thompson, J. F. *Phytochemistry 10* (1971) 1745.
9. Baur, A. H. and Yang, S. F. *Phytochemistry 11* (1972) 3207.
10. Chow, C. M., Nigam, S. N. and McConnell, W. B. *Biochim. Biophys. Acta 273* (1972) 91.
11. Mae, T., Ohira, K. and Fujiwara, A. *Plant Cell Physiol. 13* (1972) 407.

Received March 27, 1974.

The N-Terminal Amino Acid Sequence of *Bacillus subtilis* α-Amylase

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While it has been reported that native α-amylase isolated from *Bacillus subtilis* has a weight of 48 000 daltons, this weight is reduced to approximately 24 000 in the presence of 6 M guanidine.HCl, *i.e.* two subunits of equal weight are obtained.[1] Unequivocal proof that the primary structure of these two subunits is identical will have to come from sequence studies. Preliminary results, which are reported here, suggest that the enzyme molecule contains only one kind of polypeptide chain.

Two commercial preparations of the α-amylase, one manufactured by Novo Industries,** the other procured from Sigma Chemical Company and labeled Type II-A were examined. Both products exhibited identical elution volumes upon chromatography on Sephadex G-100. (Employing 0.025 M sodium acetate buffer, pH 6.0, as elution medium, a major fraction and a minor-slower migrating one were obtained. Utilizing 0.025 M ethylenediaminetetraacetate buf-

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** A gift of the enzyme by NOVO Industries is gratefully acknowledged.

fer, pH 9.0, only one peak was seen. The latter occupied the position allotted to the minor peak at pH 6.0). The amino acid composition of both samples agreed closely with that reported in the literature.[2]

In our studies with the sequencer, the two preparations yielded identical results.

For the automatic Edman degradation the Beckman sequencer (Model 890B) and the slow protein-Quadrol program was utilized.[3] Phenylthiohydantoins were identified by gas chromatography.[4]

When the intact enzyme was analyzed, the sole sequence at the amino end was found to be:

1 2 3 4 5 6 7 8
Val – Asn – Gly – Thr – Leu – Met – Gln – Tyr –
9 10 11 12
Phe – Glu – Trp – Tyr

As expected, after exposure of the enzyme to CNBr a component (A) was isolated that began as follows:

Gln – Tyr – Phe – Glu – Trp – Tyr

(The treatment with CNBr was performed by allowing a solution of 100 mg enzyme in 25 ml 70 % formic acid to stand for 17 h at room temperature followed by dilution with water and lyophilization. Chromatography on Sephadex G-100 utilizing 0.2 M ammonium hydroxide and monitored at 280 nm yielded two peaks. The first one to emerge was the fraction designated above as component A. This material seemed homogeneous upon polyacrylamide gel electrophoresis while the second fraction appeared heterogeneous).

Further studies of the primary structure of the α-amylase are in progress.

1. Mitchell, E. D., Riquetti, P., Loring, R. H. and Carraway, K. L. *Biochim. Biophys. Acta* *295* (1973) 314.
2. Junge, J. M., Stein, E. A., Neurath, H. and Fischer, E. H. *J. Biol. Chem.* *234* (1959) 556.
3. Edman, P. and Begg, G. *Eur. J. Biochem.* *1* (1967) 80.
4. Pisano, J. J. and Bronzert, T. J. *J. Biol. Chem.* *244* (1969) 5597.

Received June 12, 1974.

The Effect of *gem*-Dimethyl Groups in the Cyclization of Diynes

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Since the smallest ring obtainable by oxidative coupling of a *gem*-dimethyl substituted diyne has been shown [1] to be the 18-membered, and the possibility of making cyclic compounds with *gem*-dimethyl groups in different positions is also limited in this method, other cyclization methods had to be considered in order to prepare certain *gem*-dimethyl-substituted rings required for conformational studies.

Cyclization of terminal diynes with dibromides in liquid ammonia to give unsubstituted cyclic diynes has been described earlier [2,3] and this method has now been tried on *gem*-dimethyl substituted diynes.

The cyclization reactions are specified in Table 1 where the corresponding yields and melting points of the cyclic acetylenes formed are also given.

No cyclization to the corresponding 16-membered ring was obtained using 5,5-dimethylnona-1,8-diyne and dibromoheptane. With the same diyne and dibromononane the yield was only 6 %. The longer diyne chain, 6,6-dimethylundeca-1,10-diyne, however, reacted with the shorter dibromide, dibromopentane, to give 15 % yield of cyclic product, and with the *gem*-dimethyl substituted dibromoheptane to give a cyclic, crystalline reaction product in 59 % yield.

The results can be explained by considering the possible conformations of the reactants. Bends on the carbon chain, caused by *gauche* bonds, will most easily occur at the carbon alpha to the acetylene bond or at the *gem*-dimethyl substituted carbon.[3,4] As pointed out earlier,[1] this effect and the additional effect of the steric requirements of the *gem*-dimethyl groups, reduces the number of probable conformers for 5,5-dimethylnona-1,8-diyne to only one, shown in Fig. 1A, having the *gem*-dimethyl group at the "corner" of the chain. The terminal acetylene groups are, however, quite distant in this conformer, and they point in directions unfavourable for cyclization. This explains why no cyclization was obtained with 5,5-dimethylnona-1,8-diyne in its reaction with 1,7-dibromoheptane, as well as the low yield obtained with the same diyne and 1,9-dibromononane. With a *gem*-dimethyl group in the 4-position of dibromoheptane the dibromide chain should more easily become bent and the chances for cyclization should increase; in another work [5] the corresponding 16-membered ring was indeed obtained.

The conformational situation after extension of the diyne chain with one methylene on each