PRELIMINARY OBSERVATIONS ON THE OXIDATION OF PHLORETYLGLYCINE BY SILVER CARBONATE ON CELITE

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THE reagent silver carbonate on celite (SCC) first introduced by Fetizon[1] as a reagent for the oxidation of alcohol, was later used by Balogh *et al*[2] to oxidise 2,4,6-t-tributylphenol to blue phenoxy radical which affords the peroxide in the presence of oxygen in 90% yield. It can be expected that SCC can react with tyrosyl residues in peptides and proteins bringing about oxidative cleavage of tyrosyl peptide bonds. Evidence has been presented that the oxidation of tyrosyl residues forms the important step which leads to the cleavage of tyrosyl peptide bonds in model peptides and proteins, when the cleavage reagents such as N-bromosuccinimide[3], or electrolytic oxidations at platinum electrodes[4] are employed. In the present study the use of SCC as the cleavage reagent for the tyrosyl model peptide, phloretylglycine has been described.

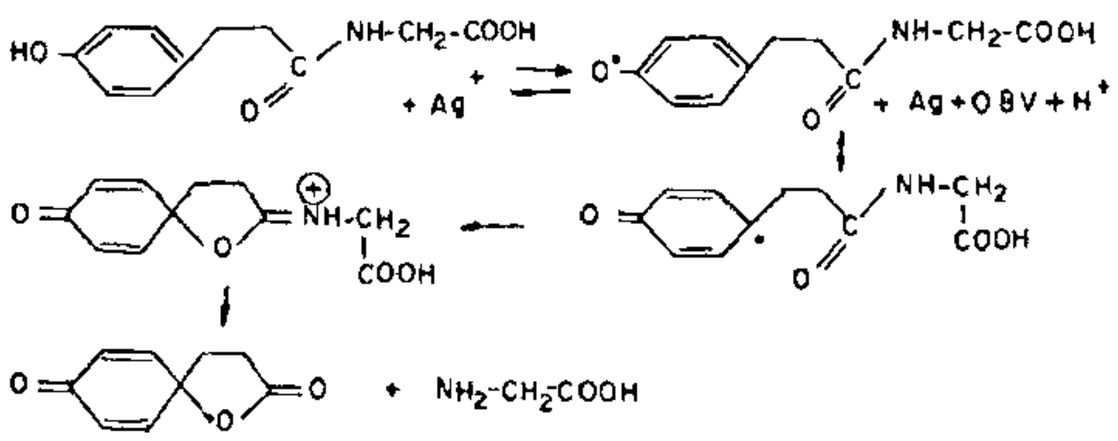
Phloretylglycine was synthesized according to the method of Schmir and Cohen[5]. SCC reagent was prepared following the method of Fetizon[1].

An aqueous solution of phloretylglycine (10 μmol/ml) in aqueous methanol or 20% acetonitrile/acetate buffer pH 4.8, was shaken with SCC reagent (100 μmol/ml) in a revolving shaker. The sample tubes were removed at intervals of 16, 24 and 48 hr. After the addition of 1 ml of 1 M sodium cyanide solution to each tube, UV absorption was measured in the clear supernatant of each sample. The reaction product was subjected to paper chromatography using the solvent system of butanol:acetic acid:water (4:1:1) when it showed a clear spot for glycine which was absent in the control sample. The reaction product was then filtered through a fine sintered glass funnel and glycine content in the aliquot of the filtrate was determined by *ninhydrin* assay.

In another experiment the SCC treated phloretylglycine (20 ml, 48 hr) was extracted with ether (3 × 25 ml) and the ether was evaporated in vacuum. The residue was taken in methanol and spotted on silica gel G plates (20 × 20 cm) and developed in benzene: methanol:acetic acid (45:8:4) solvent system[6]. A nonpolar and a polar fraction were identified by iodine spray. Both the fractions were collected from the preparative thin layer chromatographic (TLC) procedure and subjected to IR.

Exposure of phloretylglycine to the action of SCC reagent resulted in the cleavage of peptide bond and the liberation of glycine as shown by the decrease in 276 nm absorption (figure 1) as well as by the increase in the percentage of liberated glycine (figure 2). The starting phloretylglycine shows an absorption maximum at 276 nm. Its disappearance during the course of the reaction can be correlated with the modification of the aromatic ring due to oxidation. A maximum yield of 80% was obtained after 48 hours of reaction at room temperatures (25°–30°).

The high redox potential of SCC ($Ag^+ + e \rightarrow Ag$ + 0.8V) can facilitate the formation of phenoxy radical by one electron oxidation of tyrosyl moiety, thus weakening the adjacent peptide linkage which can participate in an intramolecular lactone formation leading to the cleavage of peptide bond according to the following scheme.



In agreement with this mechanism which requires the formation of dienone lactone, the IR spectrum of the nonpolar fraction isolated by preparative TLC showed the absence of the amide carbonyl absorption present in the starting material at 1630 cm^{-1} (amide I bond). The characterization of this material as dienone lactone is supported by the peaks at 1750 cm^{-1} (lactone carbonyl), 1680 cm^{-1} (conjugated carbonyl) and 1650 cm^{-1} (conjugated C=C). These values are comparable to those reported in the literature[7]. R_f value of the compound agrees well with the reported value (0.66 – 0.7) for dienone lactone obtained under the same TLC conditions[6]. The characteristic absorption maximum at 226 nm due to dienone lactone, however, could not be seen in UV which is probably due to the fact that the peak at 226 nm may become submerged in the much more intense absorption in the shorter wavelength[8].

In this context it is important to note that this cleavage being a heterogeneous one, requiring the adsorption of tyrosyl hydroxyl on celite and getting desorbed on oxidation to form phenoxy radical, the

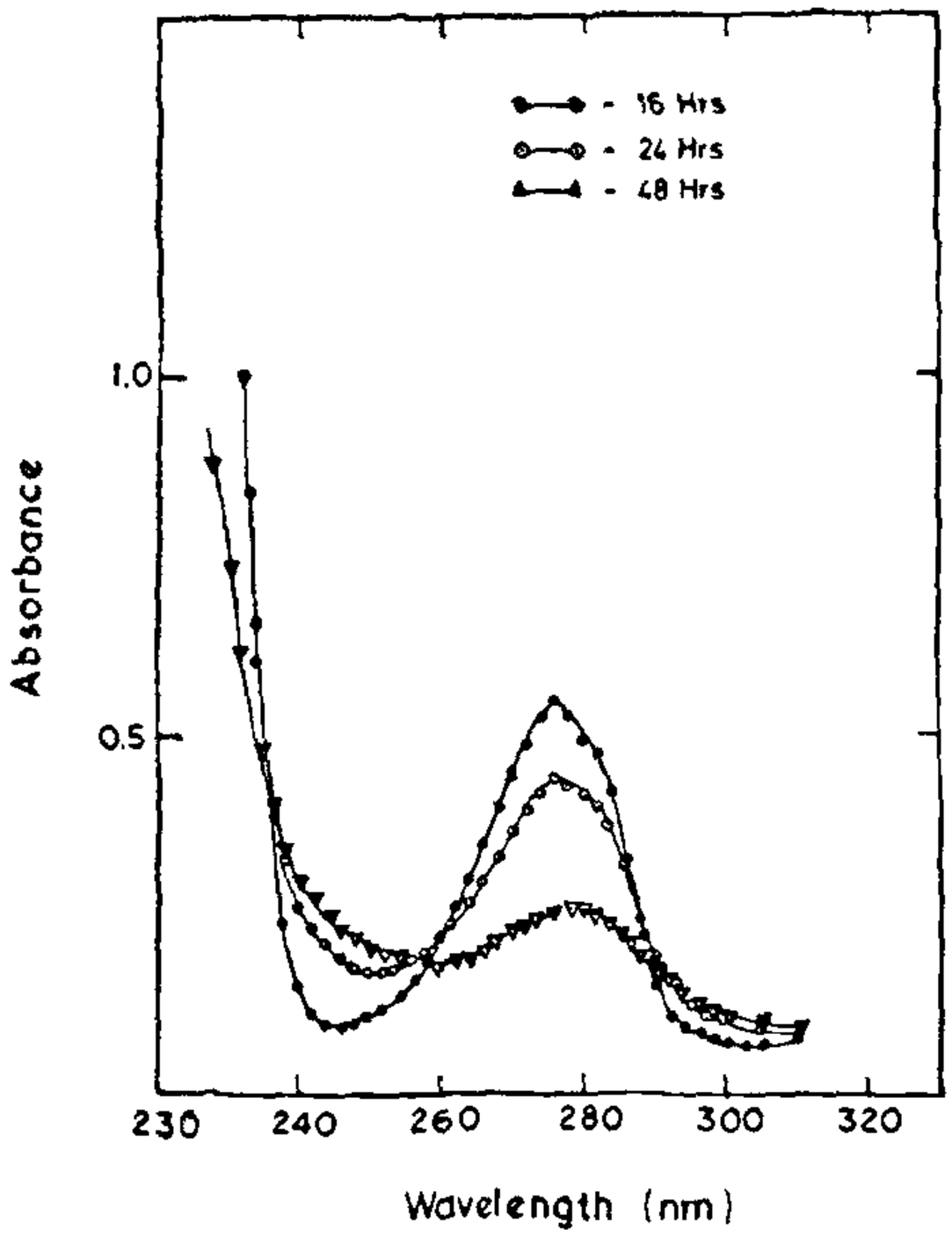


Figure 1. Changes in ultraviolet absorption of phloretylglycine treated with SCC with time.

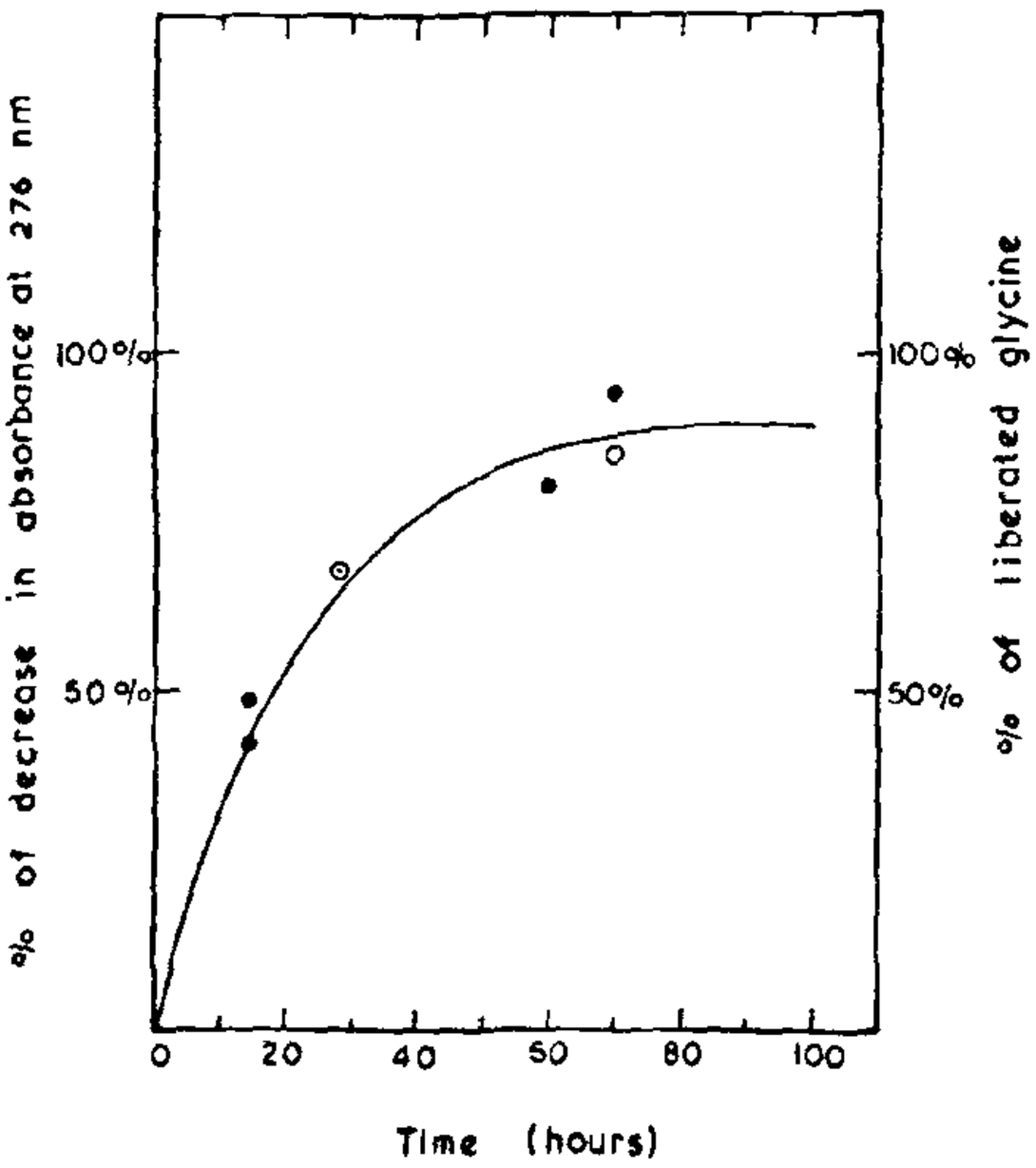


Figure 2. Correlation of loss of absorbance at 276 nm(●–●) with the liberation of glycine(⊙–⊙) in the oxidation of phloretylglycine with SCC.

degree of cleavage depends on various factors such as the amount of celite, the polarity of the solvent and the rate of agitation and temperature[9–11]. Addition of sodium cyanide probably helps in the desorption of the product after the reaction.

Similar treatment of tyrosylglycine and tyrosylalanine with SCC gave a cleavage yield of 7–8%. However the low yield of cleavage could be due to the nonprotection of N-terminal of the tyrosine moiety and subsequent formation of other intermediates[12, 13]. Application of this oxidative cleavage process to other peptides and proteins containing tyrosyl peptide bonds is possible after a study of its effect on other amino acid side chains and standardising the reaction conditions to obtain optimum cleavage yield. Work on the above lines is in progress.

The authors thank Mr. T. S. Krishnan, Acting Director, Central Leather Research Institute for kind permission to publish this work. The financial support from the US Department of Agriculture under PL 480 Grants is gratefully acknowledged.

17 July 1984; Revised 22 September 1984

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