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RESEARCH NOTE

THE U.V. IRRADIATION OF GLYCINE AND DIGLYCINE ON SILICA GEL

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RIVETT and Wilshire[1] have reported on an investigation of the u.v. irradiation of the dipeptide glycylphenylalanine on a thin layer silica gel plate, on which they subsequently detected the products by chromatography. Their finding that 2,5-diketopiperazines were produced indicated that peptide bonds can be formed in the solid state by u.v. radiation. The object of the present experiment was to determine whether peptide bonds could be formed by irradiating glycine with u.v. radiation, and to examine the competing peptide bond cleavage often referred to in the literature[2]. When glycine and diglycine were irradiated with u.v. radiation at 242 or 300 nm on pre-coated thin layer chromatography sheets, diglycine was formed from glycine, and glycine from diglycine.

EXPERIMENTAL

Aqueous solutions of diglycine and glycine (0·02 M) were prepared from British Drug Houses 'Biochemical' quality materials. These compounds were found to be homogeneous when examined by thin layer chromatography, using as solvents *n*-butanol:acetic acid:water (4:1:1 v/v) and *n*-propanol:water (4:1 v/v), and the conventional ninhydrin reagent. No attempt was made to characterise products such as *N*-acetyl amino acids or diketopiperazines, as described by Rivett and Wilshire[1].

Eastman Kodak pre-coated silica gel thin layer chromatography sheets were activated by heating to 100°C for 30 min. Small drops of 0·02 M glycine and 0·02 M diglycine were placed at separate origins on the activated sheets and allowed to dry in air. The spots of glycine and diglycine formed on the sheets were then irradiated for 48 hr with radiation of wavelength 242 or 300 nm, corresponding to the two intense peaks in the u.v. region of the super-pressure mercury lamp of a Bausch and Lomb high intensity grating monochromator. The chromatograms were developed in an Eastman Kodak 'Sandwich' chamber with *n*-propanol: water (4:1) and the ninhydrin positive products were detected by their characteristic colours with that reagent.

RESULTS

Both 242 and 300 nm radiation appeared to produce the same results. The irradiated diglycine produced a faint pink spot (when developed as described above) at an R.f.

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appropriate for glycine, giving evidence of a small amount of peptide bond cleavage. The irradiated glycine, on the other hand, produced an intense yellow stain at an R.f. appropriate for diglycine, indicating considerable peptide bond synthesis. The chromatograms also showed strongly stained spots at R.f. values corresponding to undamaged starting materials.

DISCUSSION

It is possible that the formation of diglycine from glycine on a silica gel surface may be induced by thermal processes. Rivett and Wilshire[1] studied the effect of heating glycine and phenylalanine on silica gel at 105°C for 24 hr. In their experiment, traces of glycine anhydride (2,5-diketopiperazine) and phenylalanine anhydride (3,6-dibenzyl-2,5-diketopiperazine) were detected. Thus peptide bonds can be formed by heating amino acids on silica gel. However, we consider that the products detected in our experiments were formed by photochemical processes and not thermal processes. The high resolution ultraviolet grating of the Bausch and Lomb monochromator removed all first order i.r. components and the power available to higher order i.r. spectra was reduced to negligible quantities.

The irradiation of glycylphenylalanine on silica gel thin layer chromatography sheets has been shown to result in peptide bond fission, deamination, and in the formation of 2,5-diketopiperazines[1]. The experiments outlined here have shown that readily detectable quantities of diglycine are produced when glycine is irradiated with u.v. radiation, and that peptide bond rupture also occurs but to a lesser degree. Collins and Grant[3] have noted that the u.v. irradiation of polycrystalline glycine produced very low yields of paramagnetic products. This is not surprising in view of the evidence presented here of a large yield of stable dipeptide as the end product.

This experiment has shown that peptide bonds, and possibly polypeptides, can be produced by the actinic u.v. irradiation of amino acids in the solid state.

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