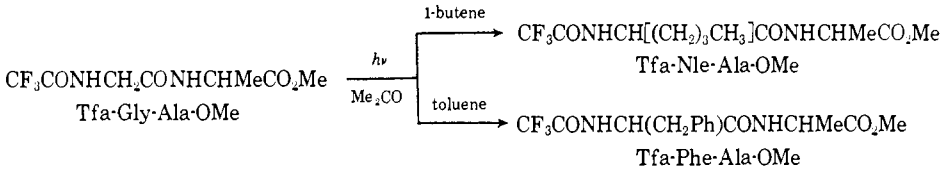
## Light-Induced Alkylation of Glycine-Containing Polypeptides[1]

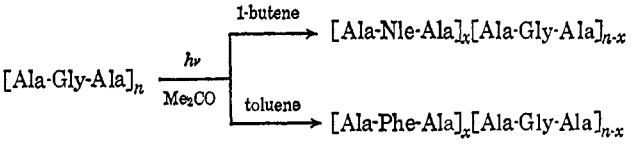
*Sir:*

A photochemical modification of glycine-containing dipeptide derivatives has been described by us recently.[2] The reactions involve an alkylation process and result in the selective conversion of a glycine residue in the dipeptide to a residue of a branched $\alpha$-amino acid. The side chain of the new amino acid residue could be preselected by the use of the appropriate "alkylating" agent. Thus, by employing 1-butene or toluene as substrates, the glycine residue in the dipeptide derivative was converted to a residue of norleucine or phenylalanine, respectively. These photochemical reactions, which were initiated with acetone, can be summarized as follows.




We report now the photoalkylation of polypeptides containing glycine and proline as well as those of glycine and alanine. These polypeptides serve as models for the study of the possible use of these reactions for the modification of glycine-containing proteins.

Sequential[3,4] as well as random[5] copolymers incorporating varying contents of glycine have been employed. Irradiation at room temperature of these glycine-containing polypeptides with 1-butene or toluene in the presence of acetone led to the conversion of glycine residues in the polypeptides to norleucine or phenylalanine, respectively. The reactions can be summarized as follows (*e.g.*, for sequential glycylalanine polypeptides)




The reactions studied and the newly formed polypeptides are described in Table I. The reactions were carried out in homogeneous as well as in heterogeneous media. In a typical experiment, a solution of 350 mg of a copolymer of glycine and DL-alanine (containing 10% glycine and 90% DL-alanine) in water (25 ml), *t*-butyl alcohol (25 ml), and acetone (15 ml) was irradiated (Pyrex filter) for 72 hr while 1-butene was bubbled through the solution. The solvents were evaporated and the residue was washed with ethyl acetate leaving a solid (320 mg) which was extracted with water. The water-insoluble fraction (120 mg) was shown to be a polypeptide consisting of alanine, glycine, and norleucine. The water-soluble fraction (200 mg) was hydrogenated over Pd–C (5%) to remove traces of unsaturation[2] and was analyzed on the amino acid analyzer. The weight-average molecular weight of the solid was determined (Yphantis method), and it was found that virtually no fragmentation of the polypeptide occurred during the reaction.

**Table I.** Alkylation Products of Glycine-Containing Polypeptides (Initiated Photochemically with Acetone)

| | Amino acid[b] composition | | | | | Product[a] Amino acid[b] composition | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Polypeptide | Ala | Gly | Pro | Mol wt | Alkylating agent | Ala | Gly | Pro | Nle | Leu | Phe |
| [L-Ala-Gly-L-Ala]$_n$ | 66.7 | 33.3 | | 3100[c] | Toluene | 68 | 30 | | | | 2 |
| [L-Ala-Gly-D-Ala]$_n$ | 66.7 | 33.3 | | 1100[d] | 1-Butene | 70.7 | 27 | | 2.3 | | |
| | | | | | Toluene | 65.5 | 31 | | | | 3.5 |
| (DL-Ala)$_m$(Gly)$_n$ | 90 | 10 | | 5100[d] | 1-Butene | 89.7[e] | 9.1 | | 1.1 | | |
| | | | | | | 91.5[f] | 8.1 | | 0.4 | | |
| | | | | | Toluene | 90.8[e] | 6.3 | | | | 2.9 |
| | | | | | | 91.1[f] | 7.7 | | | | 1.9 |
| (DL-Ala)$_m$(Gly)$_n$ | 77 | 23 | | 4800[d] | 1-Butene | 78[e] | 21.5 | | 0.5 | | |
| | | | | | | 81.9[f] | 16.5 | | 1.6 | | |
| | | | | | Toluene | 77.6[e] | 22 | | | | 0.4 |
| | | | | | | 82.5[f] | 15.3 | | | | 2.2 |
| (DL-Ala)$_m$(Gly)$_n$ | 57 | 43 | | | 1-Butene | 56.5 | 42.7 | | 0.8 | | |
| [L-Pro-L-Pro-Gly]$_n$ | | 33.3 | 66.7 | 1100[d] | Isobutene | | 30 | 67.6 | | 2.4 | |
| | | 33.3 | 66.7 | | Toluene | | 31.2 | 65.4 | | | 3.4 |

[a] In the reactions reported 7–25% of the glycine content in the polypeptides was converted to the appropriate branched $\alpha$-amino acid residues. [b] Residues per 100 total residues, determined on a Beckman-Spinco 120c amino acid analyzer. [c] Determined by the Van Slyke method. [d] Determined by the Yphantis method: D. A. Yphantis, *Ann. N. Y. Acad. Sci.*, **88**, 586 (1960). [e] Of the water-soluble product. [f] Of the water-insoluble product.

It has been shown previously[2,6,7] that in similar reactions with glycylalanine and glycylleucine dipeptide derivatives a preferential attack on the glycine residue occurs. We examined the selectivity of the process for

(1) This work was supported by National Institutes of Health Grant No. AM-10740.

(2) D. Elad and J. Sperling, *Chem. Commun.*, 655 (1968).

(3) J. P. Carver and E. R. Blout in "Treatise on Collagen," G. N. Ramachandran, Ed., Academic Press, London and New York, 1967, p 441.

(4) D. M. Segal, *J. Mol. Biol.*, in press. The author is indebted to Dr. D. M. Segal for kindly furnishing the manuscript prior to publication and for a sample of [L-Pro-L-Pro-Gly]$_n$.

(5) E. Katchalski and M. Sela, *Advan. Protein Chem.*, **13**, 243 (1958).

(6) D. Elad and J. Sperling, *Chem. Commun.*, 234 (1969).

(7) D. Elad and J. Sperling, *J. Chem. Soc., C*, 1579 (1969).

glycine residues in the glycylalanine polypeptides studied and found that the glycine residues were more reactive than the alanine residues, thus indicating that the selectivity of the process to glycine residues was preserved from the dipeptides to the polypeptides. The results of these studies are summarized in Table II.

**Table II.** Selectivity in the Photoalkylation of Polypeptides Containing Glycine and Alanine[a]

| Polypeptide | Phe:MePhe[b,d] per residue of respective starting amino acid | Nle:MeNle[c,d] per residue of respective starting amino acid |
|---|---|---|
| [L-Ala-Gly-L-Ala]$_n$ | 30:1 | |
| [(DL-Ala)$_m$(Gly)$_n$] (9:1) | 30:1 | 9:1 |
| [(DL-Ala)$_m$(Gly)$_n$] (77:23) | 15:1 | 14:1 |
| [(DL-Ala)$_m$ (Gly)$_n$] (57:43) | 8:1 | 13:1 |

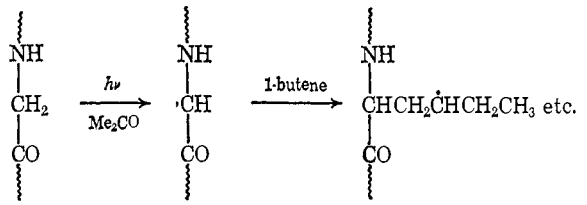
[a] Obtained by multiplying the absolute ratio of the products by the ratio of Ala:Gly in the starting polypeptide. [b] 2-Methylphenylalanine, resulting from alkylation of alanine residues with toluene. [c] 2-Methylnorleucine, resulting from alkylation of alanine residues with 1-butene. [d] The amino acid composition in the product was determined by acid hydrolysis, esterification with 1-butanol followed by trifluoroacetylation, and analysis by glpc [C. W. Gehrke and F. Shahrokhi, *Anal. Biochem.*, **15**, 97 (1966)].

The $\alpha$-carbon atoms of two adjacent amino acids in polypeptides are in a 1,4 relationship with each other.

$$(-\overset{1}{\text{NH}}\text{CHR}\overset{2}{\text{C}}\overset{3}{\text{O}}\text{NH}\overset{4}{\text{C}}\text{HRCO}-)$$

This leads to an asymmetric induction process, when one of these $\alpha$-carbon atoms is chiral, with the chiral center as the asymmetric agent. We have already shown[6] that the generation of a chiral center at the glycine residue in glycylalanine and glycylleucine dipeptides during conversion of that residue to norleucine involves the occurrence of asymmetric induction. We have found that the formation of phenylalanine in the reaction of [L-Ala-Gly-L-Ala]$_n$ with toluene involves the predominant production of the L enantiomer (70%) while the D enantiomer is the minor product (30%). It is noteworthy that in glycylalanine dipeptides hardly any asymmetric induction could be detected in similar reactions with toluene.[8] In the reaction of (L-Pro-Gly-L-Pro)$_n$ and toluene the phenylalanine formed consisted of 68% of the D enantiomer and 32% of the L enantiomer. It is assumed, therefore, that the location of the glycine residue and the conformation of the polypeptide affects the selectivity of the reactions and the asymmetric induction. Work is in progress toward the determination of these relationships.

Spectral determinations indicated that in these photoalkylation reactions acetone serves as the light-absorbing system. Product analysis indicated the involvement of free-radical intermediates.[7] The reaction sequence can thus be formulated as follows.




(8) J. Sperling, unpublished results.

The reactions of the polypeptides and toluene follow a similar path involving polypeptide and benzyl free radicals which are generated simultaneously in the reaction mixture. The detailed mechanism is discussed elsewhere.[7]

The application of these reactions for the modification of glycine-containing proteins is in progress.

**Acknowledgment.** The author is thankful to Professor Dov Elad for his interest and encouragement.

(9) This work to be submitted to The Feinberg Graduate School of The Weizmann Institute of Science for the Ph.D. in Chemistry.

**Joseph Sperling**[9]
*Department of Chemistry, The Weizmann Institute of Science*
*Rehovoth, Israel*
*Received June 16, 1969*

## Concentration Dependence of the Efficiency of Energy Transfer from $^3(\pi,\pi^*)$ Aromatic Ketones[1]

*Sir:*

Aromatic carbonyl compounds with low-lying $\pi,\pi^*$ triplet states exhibit long triplet lifetimes and low reactivity in photoreduction and Norrish type II cleavage reactions.[2-6] These properties and the high-triplet energies of these compounds make them quite attractive as sensitizers in processes in which chemical reactions of $^3(n,\pi^*)$ sensitizers are undesirable. We wish to report that the efficiency of triplet energy transfer from $^3(\pi,\pi^*)$ aromatic ketones is concentration dependent and to introduce a note of caution concerning the use of such sensitizers as probes of mechanism.

An interest in the use of $^3(\pi,\pi^*)$ sensitizers in the photoaddition of 4,4-dimethyl-2-cyclohexenone to olefins led us to examine *p*-methoxyacetophenone, *m*-methoxyacetophenone, 3,4-methylenedioxyacetophenone, and thioxanthone as sensitizers. Each of the substituted acetophenones has been shown to have a low-lying $\pi,\pi^*$ triplet.[2] The photoisomerization of 4,4-dimethyl-2-cyclohexenone to 6,6-dimethylbicyclo[3.1.0]-hexan-2-one (46%) and 3-isopropyl-2-cyclopentenone (54%) was selected as a test system.[7]

The reaction is quenched by di-*t*-butylnitroxide giving a linear Stern–Volmer plot with slope 28.5 ± 1.8 $M^{-1}$ and is sensitized by benzophenone and acetophenone. The yield of products in the sensitized rearrangement does not vary by more than 2% from the direct irradiation. The efficiency of energy transfer from these $^3(n,\pi^*)$ sensitizers is not concentration dependent. The rearrangement thus proceeds *via* a triplet excited state of 4,4-dimethyl-2-cyclohexenone. When sensitizers with low-lying $\pi,\pi^*$ triplet states were used, the efficiency of energy transfer was found to be strongly concentration dependent. The results for *p*-methoxyacetophenone, *m*-methoxyacetophenone, 3,4-methylenedioxyacetophenone, and thioxanthone are summarized

(1) Photochemical Transformations. XXXIV.
(2) N. C. Yang, D. S. McClure, S. L. Murov, J. J. Houser, and R. L. Dusenbery, *J. Amer. Chem. Soc.*, **89**, 5466 (1967).
(3) A. A. Lamola, *J. Chem. Phys.*, 47, 4810 (1967).
(4) P. J. Wagner and A. E. Kamppainen, *J. Amer. Chem. Soc.*, **90**, 5898 (1968).
(5) N. C. Yang and R. L. Dusenbery, *ibid.*, **90**, 5899 (1968).
(6) J. N. Pitts, D. R. Burley, J. C. Mani, and A. D. Broadbent, *ibid.*, **90**, 5902 (1968).
(7) O. L. Chapman, Abstracts, Twentieth National Organic Chemistry Symposium, Bloomington, Ind., June 1967, p 127; T. H. Koch, Ph.D. Thesis, Iowa State University, 1968.