isomer **7**, whose $-[{}_{\pi}2_s + {}_{\pi}2_a + {}_{\pi}2_a]$ cycloreversion to **8** is also allowed, is currently under investigation.

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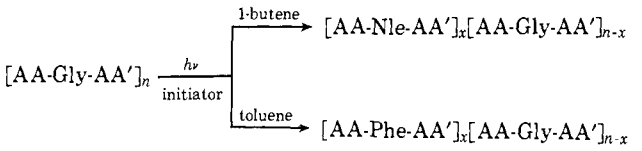
Photoalkylation of Proteins[1]

Sir:

We have recently described the photochemical modification of glycine-containing peptides.[2] This modification consists of the conversion of a glycine derivative into a branched α-amino acid derivative with a preselected side chain. The alkylation process involves substitution of an alkyl or an aralkyl group for an α-hydrogen atom in glycine. Reactions have been applied to the modification of glycine-containing di- and polypeptides, sequential as well as random, and have been found to be selective for glycine residues. The degree of selectivity depends on the location of the glycine residue in the peptide (*i.e.*, on the residue adjacent to the glycine) as well as on the molecular weight and the conformation of the peptide.[2] Examples of such reactions are those with 1-butene or toluene which lead to the conversion of glycine residues into norleucine or phenylalanine, respectively. Initiation of these photoalkylation reactions is achieved by the employment of acetone and ultraviolet light ($\lambda > 260$ nm or > 290 nm) or by a combination of biacetyl + di-*tert*-butyl peroxide (DBP) and visible light.[3] The reactions can be summarized as follows (example given for a sequential copolymer).



We wish to report preliminary results on the application of the photoalkylation reactions to the modification of glycine-containing proteins. The proteins chosen for this study vary greatly in their glycine content: collagen (from tendon; contains 33% glycine), lysozyme (chicken egg white; contains 9.3% glycine, 12 residues of a total of 129), and ribonuclease (bovine pancreatic; contains 2.4% glycine, three residues of a total of 124). In order to avoid ambiguity in the determination of the new α-amino acid produced, side chains not present in the native protein were incorporated into the glycine residues. Thus, norleucine or *p*-fluorophenylalanine, which are produced through the reaction of glycine residues with 1-butene or *p*-fluorotoluene, respectively, were chosen as the new amino acids. The initiation systems were either acetone and ultraviolet light ($\lambda > 260$ nm or > 290 nm), DBP and ultraviolet light ($\lambda > 290$ nm), or biacetyl + DBP and visible light.

In a typical experiment, a mixture of lysozyme (chicken egg white; 20 mg), water (2 ml), *tert*-butyl alcohol (3 ml), and acetone (1.5 ml) was irradiated[4] for 72 hr while 1-butene was bubbled through the mixture. The solvents were removed under reduced pressure and the residue was washed with *tert*-butyl alcohol and dried. The solid was dispersed in water and centrifuged, and the supernatant was fractionated on Sephadex G-75 with 0.1 *M* aqueous acetic acid as eluent. The fractions which exhibited the same mobility as native lysozyme were combined and freeze-dried to yield the modified protein (12 mg) having the amino acid composition[5] presented in Table I.

Table I. Photoalkylation of Lysozyme with 1-Butene Initiated with Acetone and Ultraviolet Light ($\lambda > 290$ nm)

Amino acid	Amino acid composition[a] Native lysozyme	Modified lysozyme
Lysine	5.7 (6)[b]	5.4
Histidine	1.0 (1)	0.2; 0.7[c]
Arginine	10.8 (11)	11
Aspartic acid	21	21
Threonine	7.1 (7)	6.8
Serine	9.9 (10)	9.7
Glutamic acid	5.7 (5)	5.6
Proline	2.3 (2)	2.3
Glycine	12.5 (12)	11.5
Alanine	12.5 (12)	12.3
Half-cystine	6.3 (8)	1.2; 2.0[c]
Valine	5.6 (6)	5.3
Methionine	1.5 (2)	1.5
Isoleucine	5.6 (6)	5.4
Leucine	8.2 (8)	8.1
Tyrosine	3.1 (3)	1.0; 2.3[c]
Phenylalanine	3.0 (3)	
Tryptophan	5.9 (6)[d]	Traces[d]
Norleucine		1.1

[a] Residues/molecule. [b] Figures in parentheses are according to R. E. Canfield, *J. Biol. Chem.*, **238**, 2691 (1963). [c] In the presence of phenol. [d] Determined according to J. R. Spies and D. C. Chambers, *Anal. Chem.*, **21**, 1249 (1949).

The modified lysozyme had no lytic activity on dead cells of *Micrococcus lysodeikticus*[6] and had a slightly lower electrophoretic mobility than native lysozyme on acrylamide gel.[7]

The reactions of the proteins and *p*-fluorotoluene, which led to the conversion of glycine residues into *p*-fluorophenylalanine, were conducted under similar conditions and are summarized in Table II.

Histidine, cystine, methionine, tyrosine, and tryptophan residues were sensitive to the reaction conditions

(1) This work was sponsored by National Institutes of Health, Grant No. AM-10740.
(2) J. Sperling and D. Elad, *J. Amer. Chem. Soc.*, **93**, 967 (1971).
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(4) Hanovia 450-W high-pressure mercury-vapor lamps (Pyrex filters) which were cooled internally with running water were used as the radiation source. Solutions were irradiated 3 cm from the light source in tubes 1.5 cm in diameter.
(5) Amino acid compositions were determined by hydrolysis of the protein in 6 *N* hydrochloric acid at 110° for 22 hr and analysis on a Beckman amino acid analyzer. Values are uncorrected for destruction during hydrolysis.
(6) D. Shugar, *Biochim. Biophys. Acta*, **8**, 302 (1952).
(7) R. A. Reisfeld, U. J. Lewis, and D. E. Williams, *Nature (London)*, **195**, 281 (1962).

Table II. Photoalkylation of Proteins with *p*-Fluorotoluene

Protein	Initiation system	Residues of *p*-fluorophenylalanine incorpd
Collagen	Acetone + uv	4[a]
Gelatin	Acetone + uv	9[a]
Lysozyme	Acetone + uv	0.9[b]
Lysozyme	Biacetyl + DBP + vis light	0.6[b]
Ribonuclease	Acetone + uv	0.2[b,c]
Ribonuclease	Biacetyl + DBP + vis light	0.3[b]

[a] Residues/1000 residues. [b] Residues/molecule. [c] In the presence of phenol.

and variable degrees of destruction occurred. This destruction results mostly from the interaction of the protein with the reactive species (excited ketone or free radicals[2]) generated during irradiation.[8] The undesirable destruction of these sensitive residues was reduced by the addition of free-radical scavengers, like phenol, to the reaction mixture. This resulted (in reactions initiated with acetone) in a decreased amount of destruction of sensitive amino acids without affecting seriously the amount of alkylation of the glycine residues. In lysozyme, the addition of phenol inhibited the destruction of histidine and tyrosine to a considerable extent (see Table I), but failed to prevent destruction of tryptophan. In view of the role of tryptophan residues at the binding site of the enzyme,[9] no biological activity of the reacted lysozyme could be observed. However, we could show that photoalkylation of glycine residues in ribonuclease with *p*-fluorotoluene (with acetone initiation, resulting in the incorporation of 0.12 residue of *p*-fluorophenylalanine) in the presence of phenol occurred with less destruction of tyrosine and histidine, and with retention of over 20% of the hydrolytic activity of the enzyme toward RNA.[10] Excluding phenol in similar experiments led to retention of less than 10% of the hydrolytic activity of ribonuclease (0.15 residue of *p*-fluorophenylalanine was incorporated under the latter conditions).

The detailed study of these reactions is in progress; this includes the investigation of the structural changes occurring in the proteins during the photoalkylation, the location of the modification, and attempts to find reaction conditions for minimum destruction of sensitive amino acid residues which might preserve the biological activity of the modified enzyme.

(8) In the reaction described for lysozyme, acetone absorbs *ca.* 95% of the incident light (with other initiators, light is absorbed exclusively by the photoinitiators); therefore, most of the destruction of sensitive amino acids results from reactive species generated in the reaction mixture, and not by direct interaction of the protein with light. Blank experiments indicated that the reagents do not cause denaturation of the protein in the "dark," and that inactivation of the enzyme took place only when the system was irradiated. It is noteworthy that exposure of lysozyme to ultraviolet light in the absence of the reagents, acetone, toluene, or 1-butene, resulted in as much destruction of the sensitive amino acids as during the photoalkylation process.

(9) R. A. Bradshaw and D. A. Deranleau, *Biochemistry*, **9**, 3310 (1970), and references therein.

(10) M. Munitz, *J. Biol. Chem.*, **164**, 563 (1946).

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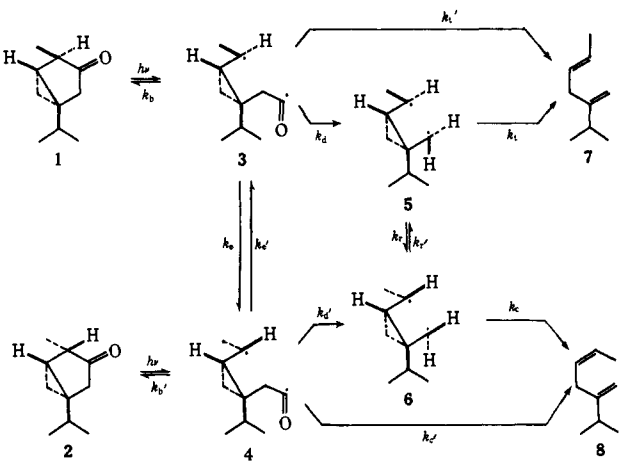
Photodecarbonylation of (−)-Thujone and (+)-Isothujone

Sir:

Photodecarbonylation of several bicyclo[3.1.0]hexan-3-one systems has been observed in vapor-[1] and solution-phase studies.[2,3] The stereochemical consequences of such transformations are of interest in light of theoretical predictions[4] concerning the course of concerted cheletropic reactions.

Distillation followed by preparative gas–liquid partition chromatography (glpc) afforded a sample of (−)-thujone[5–9] (**1**) of 99.5% purity.[10] (+)-Isothujone[5–8,11,12] (**2**) was obtained in 99.7% purity by careful distillation.



Degassed samples of 0.055 *M* 99.5% (−)-thujone or 0.062 *M* 99.7% (+)-isothujone in isooctane were irradiated at 305 nm while maintained at 30.5°. Glpc analyses indicated that a major, **7**, and a minor product, **8**, were formed in the time-independent ratio of 86.9:13.1 from either ketone. From 99.5% (−)-thujone, the amount of (+)-isothujone increased to 1.2% of the reaction mixture when 30% photodecarbonylation had occurred; from 99.7% (+)-isothujone, the amount of (−)-thujone increased to 4.0% at the same conversion. A degassed isooctane solution of 0.084 *M* cyclopentanone and 0.019 *M* **7** and **8** in the ratio 58.0:42.0 was irradiated as above without detectable change in the photoproduct mixture.

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(6) A. G. Short and J. Read, *J. Chem. Soc.*, 2016 (1938).

(7) M. S. Bergquist and T. Norin, *Ark. Kemi*, **22**, 137 (1963).

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