

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

Protein	Initiation system	Residues of <i>p</i> -fluorophenylalanine incorpd
Collagen	Acetone + uv	4 <sup>a</sup>
Gelatin	Acetone + uv	9 <sup>a</sup>
Lysozyme	Acetone + uv	0.9 <sup>b</sup>
Lysozyme	Biacetyl + DBP + vis light	0.6 <sup>b</sup>
Ribonuclease	Acetone + uv	0.2 <sup>b,c</sup>
Ribonuclease	Biacetyl + DBP + vis light	0.3 <sup>b</sup>

<sup>a</sup> Residues/1000 residues. <sup>b</sup> Residues/molecule. <sup>c</sup> 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<sup>2</sup>) generated during irradiation.<sup>3</sup> 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,<sup>9</sup> 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.<sup>10</sup> 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.

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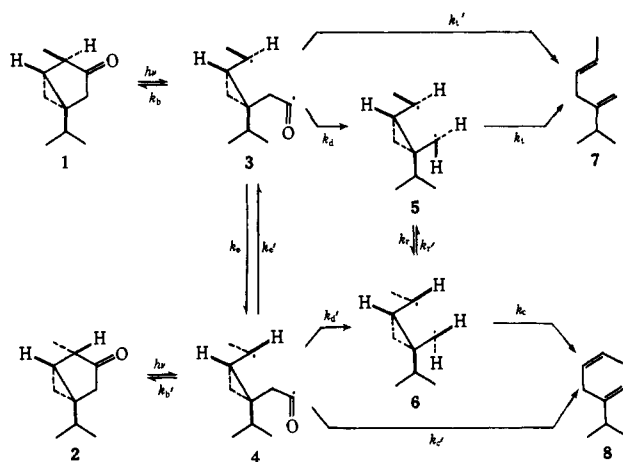
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## Photodecarbonylation of (-)-Thujone and (+)-Isothujone

Sir:

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

Distillation followed by preparative gas-liquid partition chromatography (glpc) afforded a sample of (-)-thujone<sup>5-9</sup> (**1**) of 99.5% purity.<sup>10</sup> (+)-Isothuj-



one<sup>5-8,11,12</sup> (**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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**Table I.** Variable-Temperature Photolyses

% <b>8</b> from (-)-thujone	T, °C	% <b>8</b> from (+)-isothujone
16.4 ± 0.3	89.0	17.1 ± 0.2
14.7 ± 0.1	49.8	15.4 ± 0.5
11.6 ± 0.1	10.8	11.7 ± 0.2
7.7 ± 0.1	-26.5	7.7 ± 0.1
4.4 ± 0.2	-74.5	4.8 ± 0.1

Preparative scale photolysis afforded a mixture of **7** and **8**, which were separated by preparative glpc and identified on the basis of infrared (ir) and nmr data. Compound **7**, obtained in 98.9% purity, was found to be *trans*-5-methylene-6-methyl-2-heptene<sup>2,13,14</sup> and **8**, obtained in 99.4% purity, was established as *cis*-5-methylene-6-methyl-2-heptene.<sup>15</sup>

Degassed samples of 0.125 M 99.7% (+)-isothujone or 0.068 M 99.5% (-)-thujone in isooctane were irradiated at 305 nm while maintained at various temperatures. The results, when appraised as a whole, indicate that the same mixture of photoproducts is produced by irradiation of either epimeric ketone (Table I).

A degassed sample of 0.086 M 99.6% (-)-thujone in 3-methylpentane as a glass at -196° exhibited strong, unstructured phosphorescence with an onset at 368 nm ( $E_T = 78$  kcal mol<sup>-1</sup>). When a degassed sample of 0.299 M 94.4% (-)-thujone or 0.055 M 99.7% (+)-isothujone in 3-methylpentane was irradiated at 305 nm while maintained as a glass at -196°, no decarbonylation or epimerization could be detected under conditions where 15–20% reaction occurred in liquid solution. Degassed isooctane solutions containing 0.129 M 99.7% (+)-isothujone and up to 0.10 M isoprene were irradiated in parallel at 305 nm while maintained at 30.5°. The ratio of photoproducts obtained was unchanged by the addition of triplet quencher. A Stern–Volmer plot of the data obtained at 5–8% conversion indicates a triplet lifetime

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(14) Ir (liquid film) 3080, 3020, 1640, 1378, 1362, 980, 894 cm<sup>-1</sup>; nmr (CDCl<sub>3</sub>-1%TMS)  $\delta$  1.02 (6 H, doublet,  $J = 6.5$  Hz), 1.67 (3 H, multiplet), 2.24 (1 H, broad septet,  $J = 6.5$  Hz), 2.71 (2 H, broad singlet), 4.67 (1 H, multiplet), 4.75 (1 H, multiplet), 5.46 (2 H, broad multiplet).

(15) Ir (liquid film) 3080, 3020, 1640, 1378, 1363, 893, 685 cm<sup>-1</sup>; nmr (CDCl<sub>3</sub>-1%TMS)  $\delta$  1.04 (6 H, doublet,  $J = 6.5$  Hz), 1.63 (3 H, multiplet), 2.25 (1 H, broad septet,  $J = 6.5$  Hz), 2.77 (2 H, broad doublet), 4.69 (1 H, multiplet), 4.75 (1 H, multiplet), 5.52 (2 H, broad multiplet).

of  $\tau = (3.4 \pm 0.7) \times 10^{-10}$  sec if diffusion-controlled quenching is assumed.

Attractive initial intermediates in photodecarbonylation are **3** and **4** formed by  $\alpha$  cleavage to give the more stable diradicals.<sup>16–19</sup> These species are almost certainly involved in the epimerization of the ketones.<sup>20–22</sup> In the formation of **3** and **4**, if nonadiabatic crossing from the bound triplet state at 78 kcal mol<sup>-1</sup> to an unbound state at about 75 kcal mol<sup>-1</sup><sup>23</sup> is a thermally activated process, an efficient explanation is generated for the strong phosphorescence and lack of reactivity at -196° in conjunction with the short triplet state lifetime and reactivity at higher temperatures. The existence and interconvertibility of diradicals **5** and **6** are moot.<sup>1–3,25–27</sup>

The experimental data do not require that the decarbonylation of (-)-thujone or (+)-isothujone proceed in a concerted fashion. In fact, an extremely fortuitous combination of orbital symmetry allowed pathways modulated by thermodynamic factors is necessary to explain the results in this way.

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(23) This estimate<sup>24</sup> is based on the CH<sub>3</sub>CO-CH(CH<sub>3</sub>)<sub>2</sub> bond dissociation energy and the difference in strain energy between cyclopropane and bicyclo[3.1.0]pentane.

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## Book Reviews\*

**Chemical and Biological Aspects of Steroid Conjugates.** Edited by SEYMOUR BERNSTEIN (Lederle Laboratories) and SAMUEL SOLOMON (McGill University). Springer-Verlag New York Inc., New York, N. Y. 1970. xi + 453 pp + Author Index (19 pp) + Subject Index (53 pp). \$28.00

This collaborative work represents a careful effort to review the varied aspects of steroid conjugation from the chemist's laboratory to the clinic. It follows the 1968 publication by Bernstein, Dusza, and Joseph of "Physical Properties of Steroid Conjugates."

\* Unsigned book reviews are by the Book Review Editor.

There are nine chapters, each by different authors. Each chapter is followed by its own list of references. The indices, however, are to the entire book and are extensive. In cases there are as many as three subdivision orders under a given heading, for example, "Dehydroisoandrosterone sulfate-conversion-to plasma testosterone-in canine testes." However, compounds are indexed by trivial name only, and authors frequently are to be found only in the reference listing rather than on the page to which the author index refers one.

The chapter contributors are, in each case, prominent or leading figures in this field. Judged in terms of its authors, this volume presents a remarkable range of experience and authority. One