

Figure 1. The ionization efficiency curve for the  $m/e$  130 anion from *p*-nitrobenzyl-*N*-acetylalanine.

Table I. Gas Phase Anions of Amino Acid Derivatives<sup>a</sup> (2.9 eV electrons)

Peak	Alanine- $m/e$	Valine- $m/e$	Proline- $m/e$
M - 136 <sup>b</sup>	130 (100%)	158 (87%)	157 (32%)
M - 136 - 42 <sup>b</sup>	88 (66%)	116 (7%)	—
46 <sup>b</sup>	(25%)	(100%)	(100%)
136	(11%)	(7%)	—

<sup>a</sup> The *N*-acetyl-*p*-nitrobenzyl esters of these compounds were used.

<sup>b</sup>  $m/e$  136 represents the *p*-nitrobenzyl group,  $m/e$  42 represents  $\text{CH}_2\text{CO}$ , and  $m/e$  46 represents  $\text{NO}_2^-$ ; all peaks greater than 5% of the respective base peaks are shown.

ionization efficiency (IE) curves. The IE curve for the carboxylate anion of *N*-acetylalanine ( $m/e$  130) is shown in Figure 1. This curve exhibited a dissociative resonance capture maximum at 2.9 eV which was the same as obtained for the analogous anions from the valine and proline derivatives. The IE curve also showed that the energy requirement for the efficient generation of the carboxylate anion is quite specific and a variation of less than 1 eV from the 2.9 eV maximum would cause a substantial decrease in the ion current.

It is important to note that these gas phase anions are being generated with electron energies substantially below the appearance potentials for any positive ionization of these molecules. This helps to explain the lack of extensive fragmentation and also illustrates that significant interactions are indeed occurring between low energy electrons and molecules in the positive ion mode of a mass spectrometer, even though they cannot be observed.

A wide variety of the more polar classes of bioorganic compounds such as amino acids and related compounds undergo extensive decomposition under conventional 70 eV electron impact.<sup>4</sup> In the past few years, a number of alternate ionization techniques have been developed in an attempt to circumvent this problem. The techniques more widely used include field ionization,<sup>5</sup> chemical ionization,<sup>6</sup> and more recently field desorption.<sup>7</sup> With these techniques, extensive fragmentation of sensitive bioorganic compounds is minimized and molecular ions are more easily observed.

The generation of gas phase anionic species, especially in molecules such as amino acid derivatives where extremely stable anions can be formed, has been shown to be a very low energy process. Extensive fragmentation is not observed and the anions that are produced, at least in the above examples,

can be easily correlated with the structure of the molecules. Furthermore, from a practical analytical viewpoint, ions from possible volatile contaminants such as oil, grease, plasticizers, etc. are not formed with the 2.9-eV electron energy and, thus, would not produce interfering background.

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## References and Notes

- (1) For explanations of the basic mechanisms by which low energy electrons interact with molecules please see: (a) C. E. Melton, "Principles of Mass Spectrometry and Negative Ions", Marcel Dekker, New York, N.Y., 1970; (b) L. G. Christophorou and R. N. Compton, *Health Phys.*, **13**, 1277 (1967); (c) V. Ardenne, K. Steinfeld, and R. Tummeler, "Electronenanlagerungs-Massenspektrographische Organischer Substanzen", Springer-Verlag, 1971.
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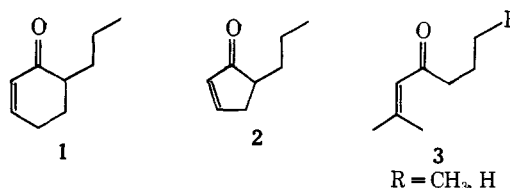
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## Photochemical $\gamma$ -Hydrogen Abstraction in an $\alpha$ -Acetylenic Ketone. Comparison with an Analogous Enone

Sir:

Irradiation of alkanones and alkyl aryl ketones frequently leads to  $\gamma$ -hydrogen abstraction followed by  $\beta$ -cleavage and cyclobutanol formation.<sup>1</sup> In the case of  $\alpha,\beta$ -unsaturated ketones, the efficiency of  $\gamma$ -hydrogen abstraction is very much lower in the six-membered enone **1** than in the five-membered analogue **2**<sup>2</sup> while the acyclic compounds **3** are photochemically inert.<sup>3</sup> Since other enone photoreactions depend on double bond rigidity<sup>4</sup> and since acyclic enones are known to undergo geometric isomerization,<sup>4,5</sup> it is reasonable that the lower efficiency of the flexible compounds is due to rapid radiationless decay induced by olefin twisting.<sup>6</sup> However, it should be added that olefin isomerization does not always preclude other reactions of excited enones.<sup>5,7,8</sup>



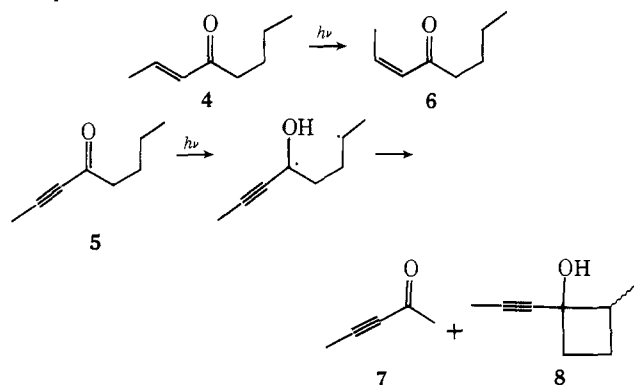
We reasoned that an  $\alpha$ -acetylenic function might have virtually no deactivating effect on the photoreactivity of the carbonyl group since rotation about the cylindrically symmetric triple bond can hardly lead to crossing of the excited state potential surface with that of the ground state. Of course, other radiationless decay pathways might operate in ynones<sup>9</sup> but since only one photochemical study of such compounds has

**Table I.** Photochemical Parameters for Several Ketones

Compound	$\Phi_{-K}^a$	$\Phi_{Cb}^b$	$\Phi_{Cl}^c$	$k_q\tau^d$	$E_T^e$	Ref
2-Hexanone	0.33, 0.5 <sup>h</sup>	0.075	0.252	50	(80) <sup>f</sup>	1, 15, 16
Valerophenone	0.42	0.091	0.33	36	74.5	17-19
4	0.49 ± 0.01	0	0	2	(73) <sup>g</sup>	This work
5	0.43 ± 0.03	0.12 ± 0.02	0.32 ± 0.03	16	73.1	This work

<sup>a</sup> Quantum yield for ketone disappearance in benzene. <sup>b</sup> Cyclobutanol formation. <sup>c</sup>  $\beta$ -Cleavage. The value for **7** is based on its calibrated GC response factor. <sup>d</sup> Stern-Volmer quenching constant of  $\Phi_{-K}$  with piperylene. <sup>e</sup> Triplet energy, kcal mol<sup>-1</sup>. <sup>f</sup> By analogy with acetone, ref 20. <sup>g</sup> By analogy with cyclic enones, ref 21. <sup>h</sup> In pentane and hexane, respectively.

appeared,<sup>10</sup> we decided that a direct comparison of an ynone with its related enone would be worthwhile. Cyclobutanol formation from an ynone could be a useful synthetic procedure because in contrast to the alkyl phenyl ketones, the product offers various possibilities for further chemical transformations.<sup>11</sup> We have therefore examined the photochemistry of compounds **4** and **5**.



Irradiation of **4**<sup>12</sup> in degassed benzene at 313 nm gave only one product according to GC. Spectral data suggested that it was *cis* enone **6** and this was confirmed by comparison with the material produced by partial hydrogenation of **5**: (**6**) ir (CCl<sub>4</sub>) 3030, 1690, 1625, 930 cm<sup>-1</sup>; NMR (CCl<sub>4</sub>)  $\delta$ , 0.93 (br t, *J* = 5.5 Hz, 3 H), 1.43 (m, 4 H), 2.04 (br, dd, *J* = 5.1 Hz, 3 H), 2.33 (br, t, *J* = 6.5 Hz, 2 H), 6.03 (br d *J* = 2 Hz, 2 H). Irradiation of **5**<sup>13</sup> (uv(cyclohexane)  $\lambda_{max}$  310 nm,  $\epsilon$  27) under the same conditions gave rise to three new GC peaks. The two closely spaced, equal area peaks at long retention time were shown by GC/MS to be isomeric with starting material while the early peak corresponded to loss of propene from **5**. Preparative GC allowed isolation of the isomeric products as a mixture and spectral data quickly pinpointed their structure as the epimers of **8**: ir (CCl<sub>4</sub>) 3610, 2240 cm<sup>-1</sup>; NMR (CCl<sub>4</sub>)  $\delta$  1.02 (br d, *J* = 6.5 Hz, 3 H), 1.81, 1.87 (s, 3 H), 1.02-2.6 (br m, 5 H); exact mass 124.0885; calcd for C<sub>8</sub>H<sub>12</sub>O, 124.0887. The early GC peak was isolated and shown to be identical with an authentic sample<sup>14</sup> of **7** (ir (CCl<sub>4</sub>) 1680, 2230; NMR (CCl<sub>4</sub>)  $\delta$  1.97 (s, 3 H), 2.17 (s, 3 H)).

It is clear from these results that enone **4** undergoes exclusive geometric isomerization while ynone **5** gives  $\gamma$ -hydrogen abstraction products, in accord with our expectations. Further experiments were aimed at defining the excited state responsible for these photoreactions. *Trans*-*cis* isomerization of **4** was hardly quenched with *cis*-piperylene (Table I) and only minor amounts of *trans*-piperylene were formed. Since many enones appear to intersystem cross<sup>6,21,22</sup> and since geometric isomerization is a characteristic triplet process, the excited state

involved here is probably a very short-lived triplet. Ynone **5** gives  $\gamma$ -hydrogen abstraction products in high chemical yield and quantum yield from the triplet state. Not only was the reaction quenchable with *cis*-piperylene, but isomerization to *trans* was readily achieved with **5** as sensitizer. Furthermore, irradiation of **5** in acetone at 313 nm gave the same products and the same quantum yields as direct irradiation.

In view of the similarity in photochemical behavior between **5** and valerophenone (Table I), it was of obvious interest to examine the phosphorescence spectrum of **5**. Strong, nicely structured emission was observed, whose 0-0 band corresponded to a triplet energy of 73.1 kcal mol<sup>-1</sup> and whose lifetime (MCIP glass -196°) was 2 ms. Thus, the analogy with valerophenone is borne out and the (n, $\pi^*$ ) state is implicated as the photoreactive one in **5**.

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## Bovine Serum Albumin as a Catalyst. 4. Identification of the Active Site

Sir:

Recently we reported in detail an unusual catalytic enzyme-like property for bovine serum albumin (BSA).<sup>1-3</sup> In the pH range of 7-9 BSA accelerates by a factor of ca. 10<sup>4</sup> the rate of decomposition of the Meisenheimer complex, 1,1-dihydro-2,4,6-trinitrocyclohexadienylate (MC). It was found from inhibition studies that the active site of the protein is also the high affinity binding site for pyridoxal 5'-phosphate (PP).<sup>2</sup>

In 1971 Anderson et al. determined the sequence of residues in the PP binding site of BSA: Ser-Leu-Phe-Glu-Lys-Pro-Lys-Lys.<sup>4</sup> The use of borohydride reduction of the Schiff base followed by amino acid analysis indicated that the penultimate lysine in the above sequence was the site of binding with PP. It seemed reasonable to us that this sequence contained the