ions rapidly react with the toluene-α-d_3 molecules to produce MDPM's before the isomerization to tropylium ions occurs. On the other hand, the tropylium ions, IV and V, are formed by the loss of D and H, respectively, *via* the symmetrical intermediate, Ib, as previously proposed by Meyerson.[4] These tropylium ions also produce the MDPM's, in which the deuterium atoms are statistically distributed in the benzyl groups, *via* the isomerization of a tropylium structure to a benzyl one.

The benzyl ions, II and III, produce the MDPM's having five and four H_{Ph}, respectively, and no H_{CH_2} in the benzyl group. When i represents the isotope effect (k_H/k_D) on the hydrogen loss from the symmetrical intermediate Ib, the ratio of IV/V is $3/5i$, and the numbers of H_{Ph} and H_{CH_2} in the benzyl group of the MDPM's produced from these ions are given by $5[(5 \times 3) + (4 \times 5i)]/7(5i + 3)$ and $2[(5 \times 3) + (4 \times 5i)]/7(5i + 3)$, respectively. On the other hand, the numbers of H_{Ph} and H_{CH_2} in the benzyl group of the MDPM's produced in the gas-phase radiolysis of toluene-α-d_3 were calculated to be 4.1 and 0.5, respectively, from the nmr and mass spectra. Thus, on the assumption of the wide range of i, 1–4,[16] the relative contributions of II, III, and IV and V to the MDPM's formation were calculated to be 30–37, 39–29%, and 31–33%, respectively, by correcting for the deuterium content of the toluene-α-d_3.[17] It may be concluded that in the gas-phase radiolysis of toluene only about one-third of the $C_7H_7^+$ ion leading to the formation of MDPM's is formed through the symmetrical intermediate, Ib. On the basis of the previous study of Howe and McLafferty,[5] the observed discrepancy between the gas-phase radiolysis and the mass spectrometry may be explained in terms of the internal energy of the molecular ion; the molecular ions having certain internal energies play important roles in the gas-phase radiolysis and produce benzyl ions before a ring expansion, and the produced benzyl ions rapidly react with toluene molecules to produce MDPM's.[18] Further studies of the reactions and formation process of the $C_7H_7^+$ ion from toluene and higher alkylbenzenes will be discussed in full papers.

Acknowledgment. We wish to thank Dr. Seung-Geon Kim for advice on the preparation of toluene-α-d_3. Thanks are also due to Yoshio Takai for assistance in the measurement of nmr spectra.

tion may be considered to be much smaller than that of the primary $C_7H_7^+$ ion, since in the gas-phase radiolysis of ethylbenzene and xylenes the C_{16} products, expected to be formed by the reactions of the analogous secondary ions, $C_6H_5C^+HCH_3$ and $CH_3C_6H_4CH_2^+$, respectively, were formed with much smaller yields than those of the benzylated products.

(16) This range of the isotope effect seems to be reasonable, since it has been reported by Howe and McLafferty that this isotope effect is 2.8 when the complete isotope scrambling occurs (see ref 5).

(17) In correcting for the deuterium content of the toluene-α-d_3, the isotope effects on the direct dissociation producing II and on the intramolecular hydride ion transfer producing III were neglected, although these isotope effects somewhat affect the calculated values.

(18) Evidence has been reported which demonstrates that some part of the isotope-scrambled $C_7H_7^+$ ion produces toluene (K. E. Wilzbach, presented before the International Atomic Energy Agency Conference, Copenhagen, Denmark, Sept 1960), although in this study no information about the $C_7H_7^+$ ion producing the products other than MDPM's was obtained.

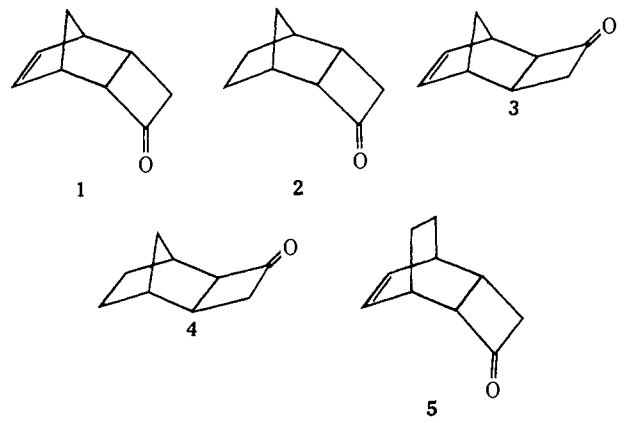
Yukio Yamamoto,* Setsuo Takamuku, Hiroshi Sakurai
The Institute of Scientific and Industrial Research
Osaka University, Suita, Osaka, Japan
Received October 9, 1971

Stereoselectivity in the Photochemical Cycloelimination of Some Polycyclic Cyclobutanones

Sir:

The solution photochemistry of cyclobutanones has been characterized by three primary processes: (1) fragmentation (cycloelimination) into ketenes and olefins, (2) decarbonylation, and (3) ring expansion to yield cyclic acetals in alcoholic solvents.[1] The cycloelimination and ring expansion reactions, which may proceed *via* a common precursor, show remarkable stereoselectivity which is accurately predicted by cleavage to generate the most stable radical pair.[1-3] Unfortunately, the selectivity of the cycloelimination, while mechanistically interesting, is often of limited synthetic potential, representing in many cases merely a reversal of the original synthesis.

The incorporation of a strained cyclic moiety, whose additional strain contribution could be removed *via* cycloelimination, into a 2,3-cis fused polycyclic cyclobutanone should repress the elimination of ketene and lead ultimately to synthetically useful products conveniently described in terms of initial cleavage to the less stable of the possible acyl–alkyl radical pairs. To test this hypothesis the photochemistry of the tricyclic ketones **1–5**[4] was examined.



The irradiation (3000 Å, 35°) of a degassed solution of **1** (0.02 *M*) in pentane resulted in the rapid disappearance of starting material and the appearance of a single major product (48–52%) together with small amounts of norbornadiene (<1%).[5] The photoproduct reacted rapidly upon addition of methanol to quantitatively produce a methyl ester (mol wt 166) assigned structure **6**. This structural assignment rests firmly on analytical and spectral data. The ir spectrum of **6** showed strong bands at ν^{film} 3000, 2900, 2800, 1740, 1635, 1260, 1190, 1160, 1020, 995, and 920 (monosubstituted olefin) and 750 cm^{-1} (cis double bond). The nmr spectrum had absorbances at τ^{CDCl_3} 3.93–

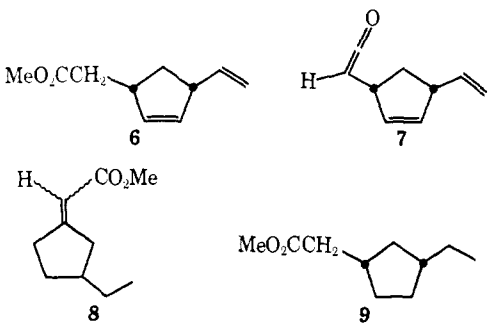
(1) (a) P. Yates, *Pure Appl. Chem.*, **16**, 93 (1968); (b) D. R. Morton, E. Lee-Ruff, R. M. Southam, and N. J. Turro, *J. Amer. Chem. Soc.*, **92**, 4349 (1970), and references cited therein.

(2) (a) N. J. Turro and D. M. McDaniel, *ibid.*, **92**, 5727 (1970); (b) N. J. Turro and D. R. Morton, *ibid.*, **93**, 2569 (1971).

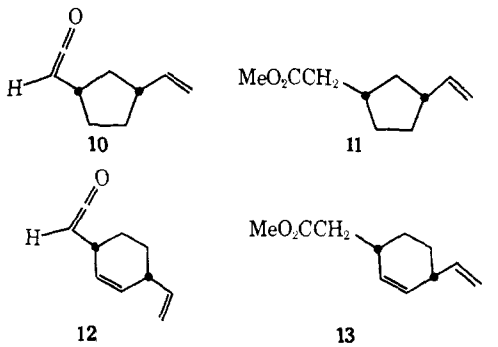
(3) The anamolous effect of a strained trans-fused cyclobutanone on the stereoselectivity of the photochemical ring expansion (although *not* cycloelimination) has been reported previously by Quinkert and coworkers: G. Quinkert, G. Cimbollek, and G. Buhr, *Tetrahedron Lett.*, 4573 (1966).

(4) The synthesis and properties of these and other polycyclic cyclobutanones will be described elsewhere.

(5) All of the cyclobutanones examined, **1–5**, produced some of the corresponding decarbonylated hydrocarbon in low yield (<2%).



4.43 (m, 3 H), 4.70–5.14 (m, 2 H), 6.24 (s, 3 H), 6.43–7.06 (m, 2 H), and 7.19–9.0 (m, 4 H). Catalytic hydrogenation (PtO_2) of **6** resulted in the rapid uptake of 2 mol of hydrogen to yield the fully saturated derivative **9**, identical in every respect with an authentic sample produced by catalytic hydrogenation of **8**.



The formation of **6** upon quenching with methanol implied that the reactive photoproduct was the alicyclic ketene, **7**. Further confirmation was produced by quenching with CD_3OD or *tert*-butyl alcohol. The former yields an ester (mol wt 170) whose nmr spectrum was very similar to **6** except that the three-proton signal at τ 6.24 was absent and the multiplet at 7.19–9.0 integrated for three protons. Consistently, subsequent quenching by *tert*-butyl alcohol produce the corresponding *tert*-butyl ester whose nmr spectrum was identical with **6** except for a *tert*-butyl singlet at τ 8.6 which replaced the methoxy singlet.

Further support for the structural assignment of the photoproduct was produced by infrared monitoring of the reaction progress. When **1** was irradiated (0.02 *M*, 35°) in methylene chloride, the carbonyl band of starting material (1775 cm^{-1}) was rapidly replaced by a strong absorption at 2155 cm^{-1} attributable to the ketene group.

The ketene **7** was reasonably stable in the photolysis solution but disappeared slowly over a period of days (25°) failing to re-form either the starting material **1** or its isomer **3**. Significantly, **3** was not found in detectable amounts in the photolysis solution even at low conversions.

Although the ultraviolet spectrum (C_6H_{12}) of **1** exhibited none of the effects generally associated with nonconjugated but geometrically proximate chromophore interaction,[6] it remained to determine the role, if any, played by the double bond in the photoselectivity of cycloelimination. For this reason, the saturated derivative **2** was investigated.

The irradiation of **2**, whose uv spectrum was essentially identical with **1**, produced the alicyclic ketene **10** as the major volatile product (43–47%), concurrently with small amounts of norbornene (<2%). A strong ketene band was again observed at 2155 cm^{-1} upon ir monitoring (CH_2Cl_2). Quenching of the photolysate produced the espected ester **11**, again in quantitative yield. The spectral data of **11** (mol wt 168) were consistently similar to **6**. The ir spectrum showed prominent bands at ν^{film} 3000, 2900, 2800, 1740, 1630, 1195, 1140, 995, and 910 cm^{-1}. The nmr exhibited absorbances at τ^{CDCl_3} 3.92–4.65 (m, 1 H), 4.9–5.53 (m, 2 H), 6.46 (s, 3 H), and 7.4–9.18 (m, 10 H). The structure of the ester **11** was further secured by the rapid uptake of 1 mol of hydrogen to produce **9**.

The spectral properties and irradiation of the exo-unsaturated isomer **3** produced some surprises. The ultraviolet spectrum (C_6H_{12}) of **3** was anomalous in that the intensity of the major vibrational component of the long-wavelength transition was *ca.* 3.7 times that of the comparable component in **1** or **2** indicating some long-range interaction between the isolated chromophores. Assignment of this intensification to double bond interaction was demonstrated by the observation that the saturated exo analog **4** showed long-wavelength components of more usual intensities (ϵ 18–28).

Irradiation of **3** (pentane) produced the ketene **7** identified by glpc comparison and quenching with methanol to produce ester **6**, together with norbornadiene (4–5%). No additional volatile products were detected in reasonable concentrations.[5] However, the yield of **7** was only 8–10% (independent of conversion). Thus, the yield of **7** from **3** was only *ca.* 20% of that produced from the irradiation of **1**.

The similarity in the starting materials and products derived from **1** and **3** rendered unlikely the possibility that **7** was produced from **3** in high yield only to be rapidly consumed by reaction with unphotolyzed starting material or its photoproducts. One alternate and intriguing possibility was that the exo geometry of the cyclobutanone with respect to the norbornene moiety was facilitating other processes involving the 2,3 bond (for example, bond cleavage and subsequent loss of ketene and/or facile ring expansion producing no isolatable products in pentane) in a manner strongly reminiscent of the enhance rates and double bond participation observed in the solvolysis of exo norbornenyl derivatives.[7] Some tentative support is drawn from the increased yield of norbornadiene from **3**. Further evidence implicating the double bond of **3** in the low yield of the ketene **7** came from examination of the saturated derivative **4**. Irradiation of **4** under identical conditions produced the corresponding ketene **9** in yields of 27–30%. The yield of the corresponding hydrocarbon fragments remained essentially unchanged. Consequently, the saturation of the double bond in **3** resulted in a three–fourfold increase of alicyclic ketene upon irradiation. In comparison, the yields of alicyclic ketene produced upon irradiation of **1** and its saturated analog **2** were quite similar.

The transition from a basic tricyclo[4.2.1.$0^{2,5}$]nonan-

(6) (a) R. C. Cookson and N. S. Wariyar, *J. Chem. Soc.*, 2302 (1965); (b) H. Birnbaum, R. C. Cookson, and N. Lewis, *ibid.*, 1224 (1961); (c) C. F. Wilcox, Jr., S. Winstein, and W. G. McMillan, *J. Amer. Chem. Soc.*, 82, 5450 (1960); (d) S. Winstein, L. deVries, and R. Orloski, *ibid.*, 83, 2020 (1961); (e) H. Labhart and G. Wagnieré, *Helv. Chim. Acta*, 42, 2219 (1959).

(7) A. Streitwieser, Jr., "Solvolytic Displacement Reactions," McGraw-Hill, New York, N. Y., 1962, p 156.

3-one skeleton, **1–4**, to the homologous tricyclo[4.2.-2.0^{2,5}]decane-3-one (**5**) renders considerable strain relief in the bicyclic moiety fused to the cyclobutanone. Consistently, the yield of bicyclo[2.2.2]octa-2,5-diene produced upon irradiation (pentane) of **5** rose to 8–10%. Surprisingly, however, no unequivocal evidence for the generation of the ketene **12** could be produced, as the ester **13** was not generated upon quenching the photolysate with anhydrous methanol. However, when **5** was irradiated in methanol solution the ester **13** (mol wt 180) was produced in 16–18% yield in conjunction with two ring-expanded isomeric (mol wt 180) acetals[8] (50%). The isolated ester **13** showed infrared absorptions at ν^{film} 3000, 2910, 2850, 1745, 1640, 1170, 1000, 920, and 735 cm^{-1}. The nmr spectrum showed resonances at τ^{CCl_4}: 3.80–4.55 (m, 3 H), 4.65–5.3 (m, 2 H), 6.32 (s, 3 H), and 6.95–8.9 (m, 8 H). At this point it is not clear whether the methanol intercepts the alicyclic ketene **12**, which for some reason is unstable in the photolysis solution, or whether the ester **13** is produced *via* a pathway other than the ketene.

In summary, we find that incorporation of additional strain in the form of a rigid bicyclic system into a *cis*-2,3-cyclobutanone fusion suppresses the high-yield regeneration of the corresponding bicyclic olefin (and presumably ketene) upon irradiation,[9] and for compounds **1–4** stereoselectively directs the cycloelimination in a manner contrary to radical stability predictions to yield synthetically useful and highly functionalized alicyclic ketenes.[10] This peculiar selectivity of cleavage was maintained, albeit to a lesser extent (ester/bicyclic olefin, *ca.* 2) in the irradiation (methanol) of **5** even though **12** was not implicated by direct observation. Additionally, the generation of alicyclic ketenes in high yield and subsequent nucleophilic quenching circumvents many of the side reactions and difficulties caused by their presence during irradiation.

The mechanistic and synthetic aspects of the chemistry of these and related cyclobutanones are under investigation.

(8) The selectivity of the photochemical ring expansion in alcoholic solvents of these polycyclic cyclobutanones will be the subject of a future publication.

(9) Bicyclo[4.2.0]oct-3-en-7-one was taken as a model compound representing a relatively strain-free cis-2,3 cyclic fusion to cyclobutanone. Consistently 1,4-cyclohexadiene was produced in high yield (60%) upon irradiation (pentane).

(10) For a brief discussion on limited useful synthetic approaches to cis-3,5-disubstituted cyclopentenes, see C. A. Grob and H. R. Pfaendler, *Helv. Chim. Acta*, **53**, 2156 (1970).

R. D. Miller,* V. Y. Abraitys
IBM Research Laboratory
San Jose, California 95114
Received September 30, 1971

The Rate-Determining Step in the Acylation of Papain by *N*-Benzoyl-L-argininamide

Sir:

Papain is the best known member of the family of sulfhydryl proteases. Although the X-ray structure of the enzyme has been reported[1] and numerous mechanistic studies have been done,[2] the mechanism of action of this enzyme[3] is much less well understood than that of the serine protease chymotrypsin.[4] In this communication we report measurements of the nitrogen isotope effect on the papain-catalyzed hydrolysis of *N*-benzoyl-L-argininamide. As in the case of the chymotrypsin-catalyzed hydrolysis of *N*-acetyl-L-tryptophanamide,[5] a substantial isotope effect is observed, indicating that carbon–nitrogen bond breaking is rate determining. Unlike the case of chymotrypsin, it seems likely that the carbon–nitrogen cleavage is entirely rate determining.

It is frequently possible to identify the rate-determining steps in enzymatic reactions by use of heavy-atom isotope effects.[5,6] In the case of chymotrypsin,[5] the presence of a substantial nitrogen isotope effect on the hydrolysis of *N*-acetyl-L-tryptophanamide indicates clearly that the carbon–nitrogen bond is being cleaved in the rate-determining step, but the variation of the isotope effect with pH indicates that the carbon–nitrogen cleavage step is only partially rate limiting.

The amide nitrogen isotope effect on the papain-catalyzed hydrolysis of *N*-benzoyl-L-argininamide was measured by a method similar to that used previously for chymotrypsin.[5] Solutions containing enzyme,[7] 0.01 *M* substrate, 0.1 *M* phosphate buffer, pH 8.00, and 10^{-4} *M* dithiothreitol were allowed to hydrolyze either to 10 or 100% of completion. Each reaction was stopped by addition of acid, protein was removed by ultrafiltration, the remaining substrate was removed by chromatography through a 5-cm column of Norit, and the product ammonia was steam distilled. The distillate was concentrated and oxidized to molecular nitrogen with hypobromite by the procedure of Bremner.[9] In control experiments it was established that enzyme, substrate, and buffers were all free of ammonia. No ammonia was formed if either substrate or enzyme was omitted from the reaction solution. The isotope effect is equal to the ratio of the isotopic composition of the 100% reaction sample to that of the 10% reaction sample, with a small correction for per cent reaction.[5]

Triplicate determinations of the nitrogen isotope effect on the papain-catalyzed hydrolysis of *N*-benzoyl-L-argininamide gave values of $k^{14}/k^{15} = 1.0227$, 1.0211, and 1.0227. This isotope effect (average value 1.022) is considerably larger than the 1.006–1.010 observed in the chymotrypsin-catalyzed hydrolysis of *N*-acetyl-L-tryptophanamide[5] and the values of 1.004–1.013 observed in reactions of amides with hydroxide ion.[10]

(1) J. Drenth, J. N. Jansonius, R. Koekoek, H. M. Swen, and B. G. Wolthers, *Nature (London)*, **218**, 929 (1968); J. Drenth, J. N. Jansonius, R. Koekoek, and B. G. Wolthers, *The Enzymes*, *3rd ed*, **3**, 485 (1971).

(2) A. N. Glazer and E. L. Smith, *ibid.*, **3**, 501 (1971); E. L. Smith and J. R. Kimmel, *The Enzymes*, *2nd ed*, **4**, 133 (1960).

(3) G. Lowe and Y. Yuthavong, *Biochem. J.*, **124**, 107, 117 (1971); G. Lowe, *Phil. Trans. Roy. Soc. London*, **B257**, 237 (1970); E. C. Lucas and A. Williams, *Biochemistry*, **8**, 5125 (1969); M. L. Bender and L. J. Brubacher, *J. Amer. Chem. Soc.*, **88**, 5880 (1966); P. M. Hinkle and J. F. Kirsch, *Biochemistry*, **9**, 4633 (1970).

(4) D. M. Blow, *The Enzymes*, *3rd ed.*, **3**, 185 (1971); G. P. Hess, *ibid.*, **3**, 213 (1971).

(5) M. H. O'Leary and M. D. Kluetz, *J. Amer. Chem. Soc.*, **92**, 6089 (1970); M. H. O'Leary and M. D. Kluetz, *ibid.*, **93**, 7341 (1971).

(6) S. Seltzer, G. A. Hamilton, and F. H. Westheimer, *ibid.*, **81**, 4018 (1959); M. H. O'Leary, *ibid.*, **91**, 6886 (1969); M. H. O'Leary, D. T. Richards, and D. W. Hendrickson, *ibid.*, **92**, 4435 (1970); M. H. O'Leary, *Biochem. Biophys. Acta*, **235**, 14 (1971); M. H. O'Leary and R. L. Baughn, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, **30**, 1240 (1971); M. H. O'Leary and R. L. Baughn, *J. Amer. Chem. Soc.*, in press.

(7) The enzyme was activated with 0.01 *M* cysteine in the presence of of 0.001 *M* EDTA. Progress of the hydrolysis of *N*-benzoyl-L-argininamide was monitored by measurement of the absorbance change at 278 nm.[8]

(8) J. R. Whitaker and M. L. Bender, *J. Amer. Chem. Soc.*, **87**, 2728 (1965).

(9) J. M. Bremner in "Methods of Soil Analysis," American Society of Agronomy, Madison, Wis., 1965, p 1256.