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).

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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.

The presence of a large nitrogen isotope effect indicates clearly that the carbon–nitrogen bond-breaking step is rate determining in the acylation of the enzyme.[11] In the case of chymotrypsin[5] we argued that the isotope effect observed is smaller than the actual isotope effect on the bond cleavage step because some prior step occurs at a rate similar to that of the cleavage step. The isotope effect varies with pH because of the variation in the relative rates of the two steps. In the case of papain, the isotope effect is so large that it is unlikely that such a reduction of isotope effect has occurred. Further, the isotope effect is larger than those observed in reactions of hydroxide ion with amides even in cases where carbon–nitrogen bond breaking is known to be rate determining.

The magnitude of the nitrogen isotope effect on the papain reaction indicates that the carbon–nitrogen bond is extensively broken at the transition state. This is consistent with the small solvent isotope effect (k_{H_2O}/k_{D_2O} = 1.35) observed in the same reaction.[8] Hydrogen isotope effects on proton transfers vary according to the position of the proton at the transition state,[12] the largest isotope effect being observed when the proton is half-way transferred, and smaller isotope effects being observed when the transition state is more asymmetric. The small solvent isotope effect here presumably occurs because the proton transfer is nearly complete at the transition state.

Thus, both nitrogen and hydrogen isotope effects indicate that the transition state in the papain-catalyzed hydrolysis of *N*-benzoyl-L-argininamide is characterized by extensive carbon–nitrogen bond breaking and concomitant nearly complete transfer of a proton from histidine to the departing nitrogen. We cannot deduce from these results whether or not a tetrahedral intermediate is involved in this reaction.

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(10) K. G. Harbison, unpublished communication.

(11) However, the presence of this isotope effect gives no information about the relative rates of acylation and deacylation.

(12) W. H. Saunders, Jr., *Survey Progr. Chem.*, 3, 109 (1966); F. H. Westheimer, *Chem. Rev.*, **61**, 265 (1961).

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Alkyl Group Isomerization in the Oxidative Addition of Acyl Chlorides to Iridium(I) Complexes

Sir:

The oxidative addition of acyl and aroyl halides to the planar dinitrogen complex $IrCl(N_2)[P(C_6H_5)_3]_2$ has recently been shown to give initially five-coordinate acyl–iridium(III) complexes which then rearrange, probably *via* an alkyl or aryl group migration, to six-coordinate alkyl- or aryl–iridium(III) species (eq 1).[1,2]

$$IrCl(N_2)(PPh_3)_2 + RCOCl \xrightarrow{-N_2} IrCl_2(COR)(PPh_3)_2 \longrightarrow IrCl_2R(CO)(PPh_3)_2 \quad (1)$$

We have independently obtained a series of these compounds by addition of acyl halides to $IrCl[P(C_6H_5)_3]_3$ (**1**)[3] (eq 2) and find that if R in the acyl halide RCOCl is branched at the α-carbon atom, the resulting alkyl–iridium(III) complex is exclusively the isomeric straight-chain derivative.

$$IrCl(PPh_3)_3 + RCOCl \xrightarrow{-PPh_3} \underset{\mathbf{2}}{\begin{matrix} & PPh_3 & \\ Cl & | & CO \\ & Ir & \\ Cl & | & R \\ & PPh_3 & \end{matrix}} \quad (2)$$

(**1**)

a, R = $CH_2CH_2CH_3$
b, R = $CH_2CH_2CH_2CH_3$
c, R = $CH_2CH_2CH_2CH_2CH_3$
d, R = $CH_2CH_2C_6H_5$

Thus, addition of 2-methylpropanoyl chloride, $(CH_3)_2CHCOCl$, to **1** in refluxing benzene gives the *n*-propyl complex (**2a**; R = $CH_2CH_2CH_3$) in 55% yield as colorless crystals: ir (CH_2Cl_2) 2030 [ν(CO)], 305, 254 cm^{-1} [ν(IrCl)]; nmr ($CDCl_3$) δ 0.02 (t, 3, CH_3, $J(CH_2CH_3)$ = 7.5 Hz), 1.10 (m, 4, CH_2CH_2). An identical product is obtained in 80% yield from **1** and butanoyl chloride. Likewise, 2-methylbutanoyl chloride, 2-ethylbutanoyl chloride, and 2-phenylpropanoyl chloride react with **1** to give the *n*-butyl (**2b**), *n*-pentyl (**2c**), and 2-phenethyl iridium(III) complexes (**2d**), respectively, in 30–40% yield. The only other iridium-containing product isolated in the first two cases is the hydride $IrHCl_2[P(C_6H_5)_3]_3$, which may arise from traces of HCl impurity in the acyl chlorides; in the case of 2-phenylpropanoyl chloride, $IrCl(CO)[P(C_6H_5)_3]_2$ is also formed (32% yield), probably as a result of facile elimination of styrene and HCl from **2d** or its precursor.

We believe that the initial product of reaction of **1** with branched acyl chlorides is the appropriate *sec*-alkyl–iridium(III) complex and that this rapidly isomerizes to the *n*-alkyl *via* a hydrido–olefin intermediate,[4] *e.g.*

$$\begin{matrix} CH_3 & & CH_3 \\ & \diagdown\ \diagup & \\ & CH & \\ & | & \\ & Ir & \end{matrix} \longrightarrow \begin{matrix} CH_3CH{=}CH_2 \\ | \\ IrH \end{matrix} \longrightarrow \begin{matrix} CH_2CH_2CH_3 \\ | \\ Ir \end{matrix}$$

Molecular models suggest that the instability of the *sec*-alkyls with respect to the *n*-alkyls may be due to unfavorable steric interaction of the branched alkyl chain with the phenyl rings of the triphenylphosphine ligands. Support for this hypothesis is provided by a reexamination of the oxidative addition of acyl chlorides to the cyclooctene complex $[IrCl(CO)(C_8H_{14})]_2$ (**3**), which gives dimeric, octahedrally coordinated, chlorine-bridged alkyl–iridium(III) complexes (**4**).[5,6] In **4** there is no obvious steric hindrance to the formation of a *sec*-alkyl complex. As previously reported,[5] 2-

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(3) M. A. Bennett and D. L. Milner, *J. Amer. Chem. Soc.*, **91**, 6983 (1969).

(4) There are many examples of reversible addition of a transition metal hydride to an olefin to give an alkyl [R. A. Schunn, *Inorg. Chem.*, **9**, 2567 (1970), and references cited therein] and there is evidence in one case that this proceeds *via* a hydrido–olefin complex: J. Chatt, R. S. Coffey, A. Gough, and D. T. Thompson *J. Chem. Soc. A*, 190 (1968); A. J. Deeming, B. F. G. Johnson, and J. Lewis, *Chem Commun.*, 598 (1970); H. C. Clark and H. Kurosawa, *ibid.*, 957 (1971).

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(6) N. A. Bailey, C. J. Jones, B. L. Shaw, and E. Singleton, *Chem. Commun.*, 1051 (1967).