the phenyl and α-methyl groups in the 1,4 biradical. The interaction between the phenyl and γ-methyl groups in the biradical from **4** should be small until the 1,4 bond is almost completely formed. The 3.1:1 trans,trans to trans,cis ratio observed for **5** can be interpreted as a superposition of the α-methyl being all trans as in **2** and the γ-methyl being 3.1:1 trans:cis as in **4**. No cis,cis isomer was detected. The 2.4:1 trans:cis ratio for **6** again reflects the small trans preference of the γ-methyl.

Transition-state arguments do not directly take into account the effects of methyl substituents upon the stability of the olefins formed upon elimination. However, in view of the high energy content of the 1,4-biradical intermediate,[6,20] ground-state stabilities would not be expected to be controlling. The lack of correlation between olefin stability and the results in Table I support this conclusion. It should be emphasized that the results in Table I are consistent with a 1,4-biradical intermediate mechanism and are not easily explained by alternate mechanisms.

The kinetic data in Table I are of interest for two reasons. First, they further illustrate the lack of correlation between triplet-state reactivity and the quantum yield for product formation.[4,6] Second, they show that the reactivity toward γ-hydrogen abstraction depends primarily upon the extent of substitution at the γ carbon and is relatively insensitive to substitution at the α or β carbons. The small increase in rate constant in the series **1**, **7**, **8** seems to reflect the increase in the number of abstractable γ hydrogens.

In conclusion, the behavior of 1,4-biradical intermediates formed by γ-hydrogen abstraction in methyl-substituted butyrophenones is highly sensitive to the position and number of substituents. Particularly important from a synthetic viewpoint are the high percentage of cyclization products formed by α-methyl aryl alkyl ketones and the stereoselectivity of the cyclization reaction. Furthermore the transition state arguments used to explain the effects of α, β, and γ substituents upon the cyclization and elimination reactions should have general applicability in predicting the behavior of 1,4 biradicals. We are currently investigating such a possibility.

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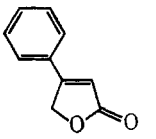
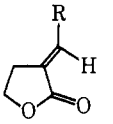
Chromophoric Lactones and the Mechanism of Chymotrypsin Action[1]

Sir:

The acylchymotrypsin intermediates formed in the chymotrypsin-catalyzed hydrolysis of esters and amides have been suggested to be in the cis (lactone-like) configuration rather than the normal ester trans configuration. This suggestion was initially made to account for the lability of the acylserine-195 bond[2] and subsequently to account for the red shifting of the λ_{max} value associated with the $\pi-\pi^*$ transition in native β-arylacryloylchymotrypsins[3] when compared to the denatured intermediate[4] and small *O*-(β-arylacryloyl)-*N*-acetylserine peptide derivatives.[5] To ascertain if the "cis hypothesis" could account for the spectral characteristics of acyl-enzyme intermediates we have compared the spectra of compounds I–IV to those for the corresponding β-arylacryloylchymotrypsins and *O*-(β-arylacryloyl)-*N*-acetylserinamides.[6]



I



II, R = C_6H_5
III, R = 2-furoyl
IV, R = 3-indolyl

The λ_{max} values for I and II (water) were found at 274 and 284.5 nm, respectively. When compared to λ_{max} for *trans*-cinnamoylchymotrypsin (Table I), the spectrum of II is seen to approach most closely that for the chymotrypsin derivative. The λ_{max} values for the exocyclic *trans*-lactones II, III, and IV are compared to those for β-arylacryloylchymotrypsins, *O*-(β-arylacryloyl)-*N*-acetylserinamides, and methyl β-arylacryloyl esters in Table I. From Table I the *O*-acylserinamides possess λ_{max} values identical with those of the corresponding denatured *O*-acylchymotrypsins, while the λ_{max} values for the lactones approach (in water) and are nearly identical (10 *M* LiCl) with those values for native *O*-acylchymotrypsins.

The reported values of ϵ_{max} for native and denatured acyl-α-chymotrypsins are however very similar (±5%).[11a] Since ϵ_{max} for a trans isomer (in a conjugated enone) is 10% > that of the corresponding s-cis isomer, it has been proposed by Oliver, *et al.*,[11b] that an s-cis → s-trans isomerization is not responsible for the difference in λ_{max} observed between the native and denatured acyl-α-chymotrypsins. The spectral shift could rather be explained as a result of the change in the polarity of the environment. However, the data of Table I show that a change in the polarity of the medium also leads to a change in ϵ_{max} for the chromophores, so that the observed spectral change for the acyl-α-

(1) This research was supported by a grant from the National Institutes of Health.

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(7) M. Sutanbawa, P. Veeravagu, and T. Padmanathon, *J. Chem. Soc.*, 1262 (1960).

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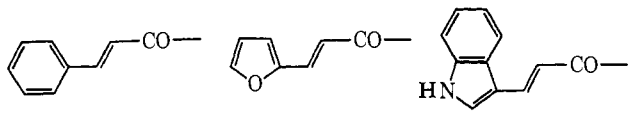
Table I. λ_{max} Values of β-Arylacryloyl Derivatives



A B C

	λ_{max}, nm ($\epsilon_{max} \times 10^4$) for acyl groups[c]		
	A	B	C
O-Acylchymotrypsins[a]			
Native (pH 4.2)	288[b] (1.78[d])	321	359 (1.78[d])
Denatured	280 (1.78[d])	309	334 (1.90[d])
O-Acyl-*N*-acetylserinamides[a]			
H_2O	281 (2.43[d])	309	335 (2.70[d])
10 *M* LiCl in H_2O	285	314	
Methyl esters			
H_2O	279	306	330
10 *M* LiCl in H_2O	280	310	335
Lactones (II, III, IV)			
H_2O	285 (2.28)	314 (2.48)	341 (2.07)
10 *M* LiCl in H_2O	288 (1.85)	320 (2.11)	349 (1.46)
EtOH	280 (2.51)	309 (2.59)	339 (2.14)

[a] See ref 4. [b] Corrected for tyrosine and tryptophan. See M. L. Bender, 143rd National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 9–14, 1962; J. F. Wooten and G. P. Hess, *Nature* (*London*), **188**, 726 (1960). [c] A, B, and C are shown above. [d] See ref 12.

chymotrypsins cannot be explained solely on the basis of medium changes.

Binding of dyes and various reporter experiments have been interpreted to show that the active site of chymotrypsin possesses very polar[12] and nonpolar[13] regions. From observations of the ^{19}F chemical shift of chymotrypsin-bound *N*-trifluoroacetylphenylalanine, Zeffren and Reavill[13b] have concluded that either the environment is of polarity approached by *ca.* 10 *M* NaCl or that the fluorine nuclei are situated adjacent to an aromatic ring and subject to its anisotropic effect. Recent nmr experiments[14a,b] provide strong evidence that cinnamate and several *N*-acyltryptophanate ions bind to native α-chymotrypsin in an identical manner, presumably at the "tosyl hole."[15] It seems reasonable to assume that the acyl groups of the chymotrypsin derivatives discussed above occupy this same location in the acylated enzymes. Examination of a model of chymotrypsin shows that this binding site encompasses regions of both polar and nonpolar character, but it is not clear which areas will be dominant in determining the ultraviolet behavior of these chromophores.

We conclude that the "cis hypothesis," if combined with the assumption of a polar binding site, might explain both the reactivity of acylchymotrypsin intermediates and the red shift noted for the β-arylacryloyl reporter groups when esterified to the hydroxyl group of serine-195. However, the cis hypothesis cannot be considered a unique explanation since the chromophore is undoubtedly held in a heterogeneous milieu and chemical or theoretical models for electronic perturbation by a heterogeneous milieu are not available.[16]

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(14) (a) J. T. Gerig and J. D. Reinheimer, *J. Amer. Chem. Soc.*, 92, 3146 (1970); (b) J. T. Gerig and R. A. Rimerman, work in progress.

(15) T. A. Seitz, R. Henderson, and D. M. Blow, *J. Mol. Biol.*, 46, 337 (1969).

(16) It should be noted that a trans-to-cis conformational change of substrate bound to chymotrypsin cannot reasonably be employed to explain the facility of the acylation step. Thus, the normal substrates for chymotrypsin are amides, and it is known that amides and lactams exhibit similar rates of alkaline hydrolysis.[17] Lactones II, III, and IV exhibit no appreciable reaction with chymotrypsin. Therefore, a cis configuration of substrate does not impart great lability of substrate to attack by chymotrypsin.

(17) M. Gordon, Ph.D. Thesis, Manchester, 1950.

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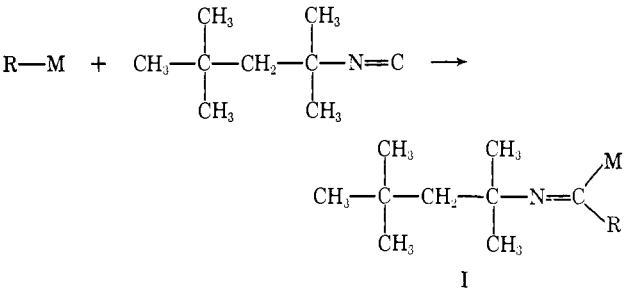
Metallo Aldimines. II. A Versatile Synthetic Intermediate[1]

Sir:

We have previously reported on the preparation of lithium aldimines and their use as intermediates for the preparation of aldehydes, C-1-deuterated aldehydes, and α-keto acids.[2] We now wish to report that aliphatic Grignard reagents[3a] also add to 1,1,3,3-tetramethylbutyl isocyanide (TMBI)[3b] to yield the corresponding metallo aldimine (I).



I

M = Li, MgBr

The aldimine I (M = MgX) is prepared by the addition of 1 equiv of TMBI to the desired alkylmagnesium halide in tetrahydrofuran.[4] Hydrolysis of I with D_2O or H_2O followed by steam distillation from a solution of oxalic acid yields the desired aldehyde in yields of 48–67%. Carbonation provides the corresponding α-keto acid. The results are summarized in Table I.

Although the yields of aldehydes and α-keto acids prepared from I (M = MgX) are lower than those prepared using the lithium aldimine reagent[2] (I, M = Li), the use of a Grignard reagent may be more expedient whenever the alkyllithium reagent is not readily available. However, we have observed that when C-1 deu-

(1) The support of this work by grants from the National Science Foundation and Public Health Service Grant No. 04064 from the National Cancer Institute is gratefully acknowledged.

(2) H. M. Walborsky and G. E. Niznik, *J. Amer. Chem. Soc.*, 91, 7778 (1969).

(3) (a) For other attempts to add Grignard reagents to isonitriles see I. Ugi and U. Fetzer, *Chem. Ber.*, 94, 2239 (1961), and references cited therein. (b) Available from Columbia Organic Chemicals, Columbia, S. C.

(4) The Grignard reagent is standardized using the method of Gilman prior to the addition of the isocyanide (H. Gilman, E. H. Zoellner, and J. B. Dickey, *J. Amer. Chem. Soc.*, 51, 1576 (1929).