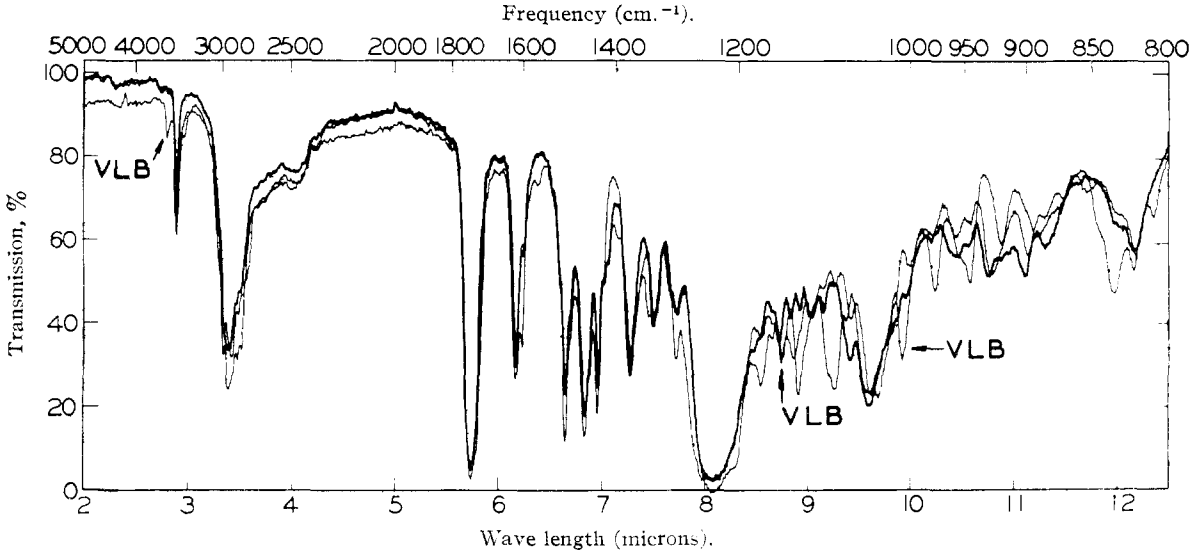

Fig. 1.— —— Leurosine; →/vincaleukoblastine; ——equimolar solution of catharanthine and vindoline.

Recently we have reported the characterization[2] and partial structures of catharanthine and vindoline, two new alkaloids from *Vinca rosea Linn.* Catharanthine was shown to be a $C_{21}H_{24}O_2N_2$, pentacyclic ester *indole* alkaloid (I) and vindoline a $C_{25}H_{32}O_6N_2$ pentacyclic *dihydroindole* compound (II). Similarities in the infrared spectra of these

I $C_{19}H_{21}N_2$ {COOCH$_3$} II $C_{20}H_{22}N_2$ {COOCH$_3$, OCOCH$_3$, OCH$_3$, OH}

two compounds and leurosine and vincaleukoblastine prompted us to use the infrared summation technique which proved successful in the deduction of structural features of reserpine[3] and other *Rauwolfia* alkaloids.[4]

Comparison of the infrared spectra of an equimolar solution of vindoline and catharanthine with that of leurosine (or vincaleukoblastine) showed an excellent agreement of wave lengths and intensities of bands in the portion of the spectra between 2.90–8.1 μ in chloroform solution.[5] This region defines the identity of the aromatic portions of the molecules (including the substitution on aromatic rings) as well as alicyclic ring systems. The oxygen functions of the monomeric alkaloids catharanthine and vindoline 2($COOCH_3$), 1($OCOCH_3$), 1(OCH_3), OH and the indole NH are present in the dimeric compounds (Fig. 1).

These assignments were corroborated by functional group analyses and n.m.r. spectra.[6] *Leurosine,* Calcd. for $C_{46}H_{58}O_9N_4$: $COCH_3$(1), 5.31; OCH_3(4), 15.31; (N)-CH_3(1), 1.85. Found: $COCH_3$, 4.91; OCH_3, 15.31; (N)-CH_3, 1.43.

Vincaleukoblastine etherate, Calcd. for $C_{46}H_{58}O_9N_4\cdot(C_2H_5)_2O$: $COCH_3$(1), 4.86; OCH_3(3), OC_2H_5(2), 20.70; (N)-CH_3(1), 1.69. Found: $COCH_3$, 4.62; OCH_3, OC_2H_5, 21.99; (N)-CH_3, 1.28.

Karrer, Schmid and co-workers have postulated the formation of dimeric curare alkaloids from either two strychnine type or two β-carboline type alkaloids bonded through C_{17} and indole nitrogen in the case of β-carboline compounds.[7]

Our results clearly indicate that another variation, namely, an indole and dihydroindole combined in a manner which leaves the indole NH free (*vide supra*) is also possible. While essential structural features of vindoline and catharanthine are clearly present in the molecules of leurosine and vincaleukoblastine, the mode of attachment remains to be elucidated. The question whether vindoline and catharanthine are precursors of these new dimeric compounds or artifacts formed during the processing of the plant material is also under study.

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DIRECT SPECTROPHOTOMETRIC EVIDENCE FOR AN ACYL-ENZYME INTERMEDIATE IN THE CHYMOTRYPSIN-CATALYZED HYDROLYSIS OF O-NITROPHENYL CINNAMATE[1]

Sir:

We wish to report the direct spectrophotometric detection of an acyl-enzyme intermediate in a

(1) This research was supported by Grant H-2416 of the National Institutes of Health.

hydrolysis catalyzed by the proteolytic enzyme, α-chymotrypsin. Spectrophotometric techniques have been utilized in studies involving oxidation–reduction enzymes, the course of reaction being followed by changes in absorption of the *enzyme* resulting from an enzyme–substrate interaction.[2] The present work exploits changes in the absorption of the *substrate*.

On the basis of kinetic studies, two catalytic steps and an acyl–enzyme intermediate have been postulated for the chymotrypsin-catalyzed hydrolysis of *p*-nitrophenyl acetate.[3,4] Degradation and inhibition studies indicate that acetylation of α-chymotrypsin by *p*-nitrophenyl acetate, phosphorylation by diisopropyl phosphofluoridate[5] and hydrolysis of acetyl-L-tyrosine ethyl ester[6] occur at the same position in the enzyme. Acetylchymotrypsin has been isolated[7] and its deacylation shown to be inhibited by 8 *M* urea.[8]

In contrast to previous studies, we have utilized an ester whose acid and alcohol components could be detected spectrophotometrically. At *p*H 7, *o*-nitrophenyl cinnamate (λ_{max}, 287 mμ; ϵ_{max}, 2.38×10^4) gives on hydrolysis cinnamate ion (λ_{max}, 268 mμ; ϵ_{max}, 1.93×10^4) and *o*-nitrophenol (λ_{max}, 360 mμ; ϵ_{max}, 2.53×10^3) (λ_{max}, 279 mμ; ϵ_{max}, 5.61×10^3). Approximately equimolar amounts ($0.42 \times 10^{-4} M$) of ester and α-chymotrypsin reacted in phosphate buffers containing 10% acetonitrile at 25° at *p*H's 5.48 to 8.24. Thus, at *p*H 6.2, these spectrophotometric observations were made: (1) at 350 mμ, the liberation of *o*-nitrophenol is practically complete in one minute; and (2) at 250 mμ, the absorption decreases reaching a minimum in about one minute; the absorption then rises to a maximum value in about 40 minutes. The absorption at infinity is the sum of *o*-nitrophenol and cinnamic acid absorptions (Fig. 1). Since cinnamic acid is not appreciably formed by the time *o*-nitrophenol formation is complete, the decrease in absorption at 250 mμ must correspond to the formation of a cinnamoyl-chymotrypsin intermediate.

The spectrum of cinnamoyl-chymotrypsin was obtained at lower *p*H's (5.48 and 4.5) to avoid complications arising from the formation of cinnamic acid. Cinnamoylchymotrypsin has a single peak at 293 mμ (ϵ_{max}, 1.6×10^4) which corresponds more closely to that of cinnamoyl esters (λ_{max}, ~285 mμ) than to cinnamoylimidazole (λ_{max}, 307 mμ). Cinnamoylchymotrypsin was isolated by Balls'[7] method and gave a spectrum similar to the above; its rate of deacylation at *p*H's 7.0 and 8.2 corresponded to reactions run directly at those *p*H's. The rates of acylation and deacylation of cinnamoyl-chymotrypsin depend on groups whose pK_a's are about 6.6 and 7.4, respectively, results similar to those obtained previously.[4]

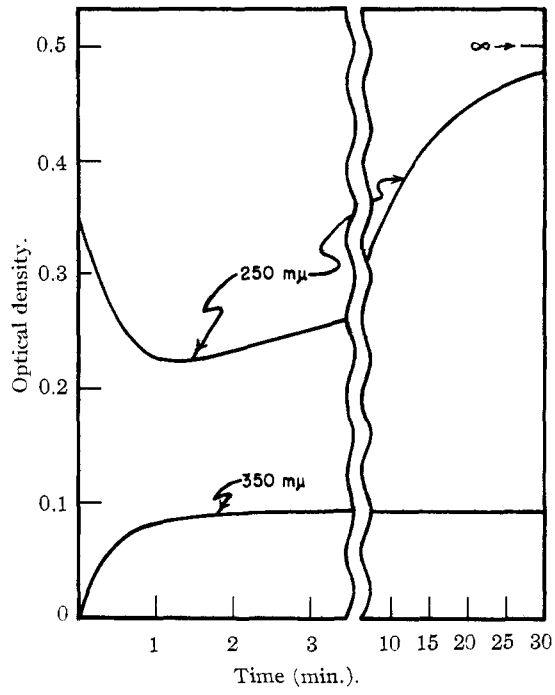

Fig. 1.—The α-chymotrypsin-catalyzed hydrolysis of *o*-nitrophenyl cinnamate at *p*H 6.2 at 25° in phosphate buffers containing 10% acetonitrile. Measurements were taken on a Beckman DK-2 recording spectrophotometer: $E = S \cong 0.42 \times 10^{-4} M$.

The technique of labeling the active sites of enzymes by highly absorbing groups should help elucidate the mechanisms of enzymatic catalysis.

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THE SYNTHESIS OF BIS-TRIPHENYLCYCLOPROPENYL, AN UNDISSOCIATED DIMER OF THE TRIPHENYLCYCLOPROPENYL RADICAL, AND ITS ISOMERIZATION TO HEXAPHENYLBENZENE

Sir:

In view of the striking contrast in the cyclopropenyl series between derivatives of the stable cation[1] and those of the unstable anion[2] it seemed desirable to obtain evidence on the stability of a cyclopropenyl radical. Accordingly we have reacted *sym*-triphenylcyclopropenyl bromide[1] (I) with zinc dust[3] in benzene and obtained (66%) the dimer (II), m.p. 225–226° (with rapid resolidification to an isomer, m.p. 430°). Found: C, 94.40; H, 5.57. The ultraviolet spectrum, λ_{max} 306 mμ (log ε 4.5) with shoulders at 290 and 320 mμ, is similar to that of other diphenylcyclopro-

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