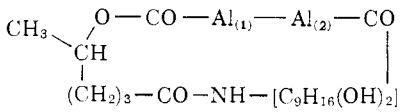
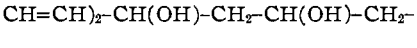
incorporation into the lactone, **3** may now be represented as the diacetate of **5**.



5

Hexahydrogriseoviridin diacetate (**6**),[2] on alkaline hydrolysis, gave 2-mercapto-5-hydroxyhexanoic acid. This establishes one point of attachment of the cysteine moiety.[8] Hydrolysis of **1** (diacetate) with hydriodic acid and red phosphorus gives cystine[2]; **6** behaves similarly. Prior reduction of the lactone function with lithium borohydride gave, after hydrolysis, no cystine or cysteine. Alanine$_{(1)}$ in **5** is thus derived from the cysteine precursor.

Griseoviridin diacetate gave a maleic anhydride adduct (m.p. 266–269°) under mild conditions. Oxidation of griseoviridin itself with manganese dioxide gave a conjugated dienone (m.p. 245–248°; λ_{max} 277 mμ (ϵ 20,100)). The dienone monoacetate (m.p. 238–241°) was hydrogenated (palladized charcoal) and the product (m.p. 173–175°; no high intensity absorption above 225 mμ) on treatment with 0.023 *N* sodium hydroxide for 3 seconds lost the remaining acetoxyl group. A solution of the product exhibited maximal absorption at 385 mμ (ϵ 23,000) displaced to *ca.* 255 mμ in neutral and acidic solution; this shift (vinylogous β-ketoamide) was reversible.[9,10] Ozonolysis of griseoviridin diacetate and mild acid hydrolysis gave glycine as the only amino acid, whereas omission of the ozonolytic cleavage led to no trace of glycine. Identical observations were made on the derived dienone. The C_{10} fragment is therefore to be represented as



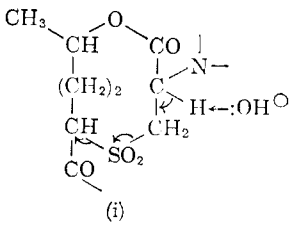
The precursor of alanine$_{(2)}$ in **5** has the following properties. It contains ethylenic unsaturation which gives rise to a band at 1625 cm.$^{-1}$ in the infrared. The terminal carbon atom bears a single hydrogen atom, and, since this is responsible for a singlet at τ 1.9, the carbon atom bearing the proton must be attached, taking into consideration the disposition of the remainder of the molecule, to an oxygen or nitrogen atom not bearing hydrogen. On alkaline hydrolysis the fragment gives rise to one molecule of formic acid and to glycine. On Raney nickel and other catalytic reduction, it suffers hydrogenolysis to give alanine$_{(2)}$.[11] These facts are mandatory for the grouping —CO—C(—N<)=CH—Y.

If Y, here, be oxygen one of the amide carbonyl functions (as enol) must be involved. All such substances would, however, be basic, the weakest being the 4-carboxamidoöxazole formed by utilizing the amide carbonyl of the C_{10} fragment. This possibility is excluded because, first, 4-carbethoxyoxazole is titrable with perchloric acid in acetic acid, whereas under the same conditions a griseoviridin diacetate solution is indistinguishable from the solvent, and, secondly, all griseoviridin derivatives *including* **5** have an identical amide intensity in the 1660 cm.$^{-1}$ region.[12] Y cannot, therefore, be oxygen.

Active hydrogen determinations on griseoviridin diacetate by modified Zerewitinoff (lithium aluminum hydride), by quantitative infrared measurements on deuterated and undeuterated material, together with equivalent n.m.r. estimations, clearly established the presence of only two such functions. Y hence must be nitrogen. Implication of the cysteinyl nitrogen or that of the other C_3 fragment must be denied because of the requirements of unsaturated small rings which are incompatible with the general properties of griseoviridin and its derivatives.[13] The structure **1** follows.[14]

We wish to thank the National Research Council of Canada and the National Institutes of Health for supporting this work.

(8) Indirect evidence is also available. The sulfone of **6** obtained with perbenzoic acid, on hydrolysis with alkali, gives 5-hydroxyhexanoic acid and sulfur dioxide. This can be rationalized as in (i): a process not available to **6** itself.



(i)

(9) The absorption maximum at 255 mμ is a "difference" spectrum between the eliminated and non-eliminated products, and its precise location and extinction, therefore, are subject to some error.

(10) Since, of the three actual or potential hydroxyl groups in griseoviridin, two have now been shown to be present in the C_{10} fragment the participation of the third, that in **2**, in the lactone is thus established.

(11) The "hydrogenolysis" presumably occurs by reduction, β-elimination and further reduction.

(12) This possibility is also excluded by the transformations and properties of "griseoviridin" hydrochloride[2] to be reported shortly.

(13) Amongst these may be mentioned the resistance of the relevant ethylenic linkage to hydrogenation and to acid.

(14) The occurrence of etamycin[15] (viridogrisein[1,2]) together with grieseoviridin is reminiscent, in general terms, of the ostreogrycin complex.[16]

(15) J. C. Sheehan, H. G. Zachu and W. B. Lawson, *J. Am. Chem. Soc.*, **79**, 3933 (1957).

(16) A. Todd, *Intern. Symp. Org. Chem. Nat. Products* (I.U.P.A.C.), Brussels, 1962.

M. CATHERINE FALLONA
DEPARTMENT OF CHEMISTRY T. C. MCMORRIS
UNIVERSITY OF WESTERN ONTARIO P. DE MAYO
LONDON, ONTARIO T. MONEY
CANADA A. STOESSL

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INVERSION OF ANTIPODAL REACTIVITY IN HYDROLYSIS OF ETHYL α-ACETOXYPROPIONATE BY α-CHYMOTRYPSIN[1,2]

Sir:

In our study of structural requirements for stereospecificity in hydrolysis of esters by α-chymotrypsin we have found that the presence of an alpha or beta acetamido group at a center of asymmetry or at a developing center of asymmetry results in

(1) We are pleased to acknowledge support of this work by the Division of Research Grants, The National Institutes of Health, RG4584.

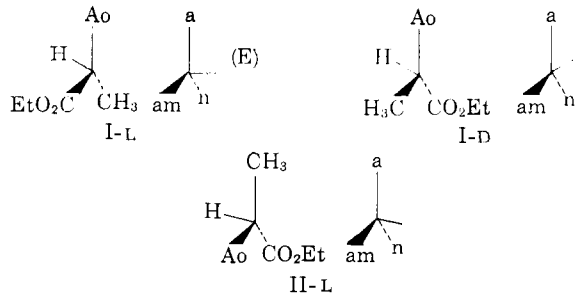
(2) For preceding paper in this series see S. G. Cohen and E. Khedouri, *J. Am. Chem. Soc.*, **83**, 4228 (1961).

stereospecific hydrolysis.[3] In a variety of these substrates, of the asymmetric type Cabde and of the symmetric type Cabdd, the absolute steric sense of the hydrolysis, where determined, was[2] L. We are studying the effect of the alpha and beta acetoxyl substituent in place of the acetamido group in this reaction, and wish to report an inversion of antipodal reactivity in the hydrolysis of ethyl α-acetoxypropionate, $CH_3CH(OCOCH_3)$-$CO_2C_2H_5$.

Ethyl *dl*-α-acetoxypropionate was subjected to the action of α-chymotrypsin, 12 mg./ml., at pH 7.8 in a pH-stat for 12 hours, hydrolysis stopping after about 50% reaction. Unhydrolyzed ester was recovered in 85% yield, α_{obsd} $-2.32°$, $[\alpha]^{22}D$ $-22°$, 5.3% in chloroform. Negative rotation also was observed in acetone, ethyl acetate and in ethyl *dl*-α-acetoxypropionate. Since the L-ester has negative rotation,[4] $[\alpha]^{22}D$ $-48°$, this indicates more rapid hydrolysis of the D-enantiomorph than the L from the racemate, and by a ratio of about 2.7 to 1. The product of hydrolysis, α-acetoxypropionic acid, was isolated from the hydrolysate in 85% yield α_{obsd} $+ 1.77°$, $[\alpha]^{22}D$ $+ 23.3°$, 3.8% in chloroform; it was characterized as the substituted ureide from 1,3-bis-(*p*-dimethylaminophenyl)-carbodiimide, m.p. 149–151°, $[\alpha]^{22}D$ $-17°$. *Anal.* Calcd. for $C_{22}H_{28}O_4N_4$: C, 64.06; H, 6.84; N, 13.58. Found: C, 63.94; H, 6.95; N, 13.62. D-α-Acetoxypropionic acid has positive rotation,[5] $[\alpha]^{22}D$ $+ 49°$, and this confirms the more rapid hydrolysis of the D-enantiomorph by a ratio of about 2.8 to 1.

Since such experiments with racemates may lead to results different from those found in study of the individual enantiomorphs[6a,b] the D(+) and L(−) ethyl α-acetoxypropionates were prepared and hydrolyzed separately by α-chymotrypsin, 5 mg./ml. at pH 7.2 in 0.1 *N* NaCl. The initial zero order rates of hydrolysis were determined at several concentrations, the first numbers in each set being the concentration, the second the rate: L: 2.84×10^{-3} *M*, 0.646×10^{-7} mole/l./sec.; 4.30, 0.800; 6.67, 0.969. D: 2.99×10^{-3} *M*, 1.08×10^{-7} mole/l./sec.; 5.26, 1.27; 6.19, 1.76; 7.34, 2.38. The separate enantiomorphs also lead to more rapid hydrolysis of the D compound, with the ratio in rates approaching a value in excess of 2 with increasing concentration of substrate, consistent with the results of the isolation experiments, which had been carried out on saturated solutions of the racemate. The data indicate that the L-enantiomorph has a more favorable K_m and a less favorable k_3; the absolute values of these kinetic parameters will require more extensive kinetic experiments. Enzymatic hydrolysis of the ethyl L-α-acetoxypropionate in a preparative experiment led to L-α-acetoxypropionic acid, characterized as its ureide derivative from 1,3-bis-(*p*-dimethylaminophenyl)-carbodiimide, m.p. and mixed m.p. with an authentic sample, 146–147°, $[\alpha]^{22}D$ $+ 48.6°$, 2.22% in chloroform.

We suggest that ethyl α-acetoxypropionate may associate as an extended tetrahedron with α-chymotrypsin in two conformations. In both, the α-hydrogen assumes its normal orientation, presumably fitting into a restricted space, determining the sense of approach of the other three groups to the enzyme, E. In one conformation, which is preferred, I-L and I-D, the acetoxyl group (Ao), lacking the polar N–H of a typical acetamido substrate, associates with the non-polar site (a)



of the enzyme at which the β-aryl groups of the natural substrates[7] normally associate. The L-enantiomorph does this more effectively than the D, but only with the D enantiomorph does this association place the ester group near the nucleophilic site (n) which leads to hydrolysis. In the second, somewhat less favored mode of association, II-L, the acetoxyl group associates with the acylamido site (am) and this leads to hydrolysis of the L enantiomorph, but not of the D. A similar analysis of the inversion of antipodal reactivity in the hydrolysis of 1-keto-3-carbomethoxytetrahydroisoquinoline,[8] indicates that the benzamido moiety in that substrate may be associating with α-chymotrypsin at the β-aryl site, the phenyl group being dominant in effecting association, leading to a rotation of 120° and a situation similar to that of I-D, as has been proposed by Hein and Niemann.[9]

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(4) J. Kenyon, H. Phillips and H. G. Turley, *J. Chem. Soc.*, **127**, 399 1925).

(5) C. M. Bean, J. Kenyon and H. Phillips, *ibid.*, 303 (1936).

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(7) H. Neurath and G. W. Schwert, *Chem. Rev.*, **46**, 69 (1950).

(8) G. E. Hein, R. B. McGriff and C. Niemann, *J. Am. Chem. Soc.*, **82**, 1830 (1960).

(9) G. E. Hein and C. Niemann, *Proc. Natl. Acad. Sci.*, **47**, 1341 (1961).

SAUL G. COHEN
JOHN CROSSLEY
EZRA KHEDOURI
ROBERT ZAND
DEPARTMENT OF CHEMISTRY
BRANDEIS UNIVERSITY
WALTHAM 54, MASSACHUSETTS

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RADON FLUORIDE[1]

Sir:

Shortly after Bartlett[2] reported the reaction of xenon with platinum hexafluoride, Claassen, Selig and Malm[3] prepared xenon tetrafluoride by direct combination of the elements. We have studied the reaction of trace amounts of radon with fluorine and found that radon forms a stable fluoride which is less volatile than XeF_4.

Gaseous radon (Rn^{222}), collected from an aqueous solution of radium chloride, was passed through

(1) Based on work performed under the auspices of the U. S. Atomic Energy Commission.

(2) N. Bartlett, *Proc. Chem. Soc.*, 218 (1962).

(3) H. H. Claassen, H. Selig and J. G. Malm, *J. Am. Chem. Soc.*, **84**, 3593 (1962).