Although removal was complete in 8 hr., traces of octapeptide could still be found in tryptic digests after 4 hr. of treatment of the modified protein with NH_3-NH_4 acetate. A single study indicated that decreasing the reagent concentration slowed the reaction, as expected.

To confirm the absence of side reactions accompanying the reversal of amidination, both oxidized insulin and modified oxidized insulin were treated for 8 hr. at pH 11.3 with the ammonia reagent and aliquots digested with trypsin. Electrophoretic patterns of samples exposed to the reagent were compared with those of oxidized insulin and trypsin-treated oxidized insulin by staining with hypochlorite[13] and with reagents specific for tyrosine, arginine, and histidine.[14] None of the samples subjected to ammonia treatment displayed any bands not present in the corresponding untreated samples.

If, however, the acetimidyl group was removed by treatment with concd. ammonia at 100° for 10 min.,[15] the N-terminal B chain peptide was completely converted to another material of greater mobility.[16] After 8 hr. of reaction with concd. ammonia at room temperature or after 10 min. of heating at 100° in NH_3-NH_4 acetate mixtures at pHs 13, 12, or 11.3, partial conversion of the B chain peptide to side product occurred. The conditions finally chosen, pH 11.3 and room temperature, were mild enough to prevent this side reaction.

Hydrazine, 0.6–1.2 *M*, adjusted to pH 9 by the addition of acetic acid, displaced the acetimidyl group from the lysyl residue of acetamidino oxidized insulin in 24 hr. at room temperature. However, the absence of concomitant side reactions has not been fully established.

It thus appears feasible to regenerate completely the amino groups from large amidinated peptides. The tryptides included within the sequence of each large peptide may then be obtained by redigestion of the isolated unmasked peptides. The acetimidyl blocking group possesses some convenient properties. Essentially complete modification of amino groups can be obtained,[8] minimizing the number of peptides appearing in tryptic digests; the derivatives approximate the charge and solubility of the original proteins, unlike carbobenzoxy[4] or trifluoroacetyl[6] derivatives, which are much less soluble, especially in acid solution; and the relatively great stability of the amidino derivatives permits the separation of the large modified peptides by a variety of procedures. Moreover, since the degree of removal can be controlled by an appropriate choice of time and reagent concentration, redigestion of a partly unmasked peptide with trypsin can yield a mixture of peptides from which the alignment of tryptides comprising the large peptide may be deduced.

The present preliminary work on insulin therefore suggests that amidination may be generally useful in sequence determinations on larger proteins.

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(15) K. Satake, M. Tanaka and H. Shino, *J. Japan. Biochem. Soc.*, **50**, 6 (1961).

(16) E. J. Harfenist and L. C. Craig, *J. Am. Chem. Soc.*, **75**, 5528 (1953); F. Carpenter, *J. Biol. Chem.*, **237**, 404 (1962).

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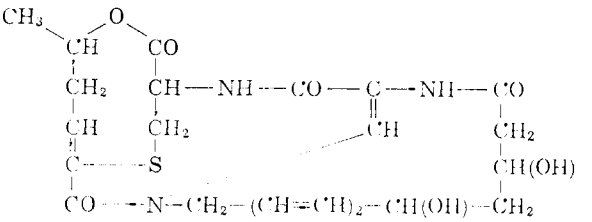
RECEIVED AUGUST 31, 1962

GRISEOVIRIDIN

Sir:

The isolation[1] and characterization[2,3] of griseoviridin, an antibiotic from a strain of *Streptomyces griseus*, have been reported previously. We now wish to present evidence leading to the assignment of the structure (1) to griseoviridin.



1

Griseoviridin has the molecular formula $C_{22}H_{27}N_3O_7S$.[2,4] It contains two acylable hydroxyl groups,[2] and on acid hydrolysis gives cystine[2] and 2-oxo-5-hydroxyhexanoic acid.[3] The latter is itself derived from the six carbon fragment: $CH_3CH(—O—)—CH_2—CH=C(—X—)—CO—$ (2) where X is O, N or S.[3] On reduction with Raney nickel the diacetate gives the saturated perhydrodethiogriseoviridin diacetate (**3**).[2] Acid hydrolysis of this gives two molecules of alanine, whilst reductive hydrolysis[2] leads to ω-aminodecanoic acid as the only other identified product. All carbon atoms in griseoviridin are thus accounted for.

Alkaline hydrolysis of **3** (uptake of three equivalents) and esterification (diazomethane) gave a neutral methyl ester, (**4**) (m.p. 125–127°).[5] Since no fragment is lost during the hydrolysis **3** is thus a lactone.

Reduction of **4** with lithium borohydride[6] followed by acid hydrolysis, neutralization and steam distillation gave alaninol.[7] Mild acid hydrolysis of **3** gave alanylalanine. Since, as will be shown, only the hydroxyl group in **2** is available for

(1) Q. R. Bartz, J. Standiford, J. D. Mold, D. W. Johannessen, A. Ryder, A. Maretzki and T. H. Haskell in "Antibiotics Annual" 1954–1955, Medical Encyclopedia, Inc., New York, N. Y., 1955, pp. 777–783.

(2) D. E. Ames, R. E. Bowman, J. F. Cavalla and D. D. Evans, *J. Chem. Soc.*, 4260 (1955); D. E. Ames and R. E. Bowman, *ibid.*, 4264 (1955); 2925 (1956).

(3) P. de Mayo and A. Stoessl, *Can. J. Chem.*, **38**, 950 (1960).

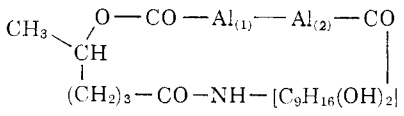
(4) Knowledge of the exact number of hydrogen atoms was not revealed by analyses, but awaited the final elucidation of structure.

(5) Satisfactory analyses have been obtained for all new compounds reported except those used without further purification. Substances have been characterized by ultraviolet, infrared, and, in most cases, n.m.r. spectra which are in accord with the assigned structures.

(6) Under the conditions used the ester carbonyl was reduced whilst the infrared spectrum indicated no reduction of the amide functions.

(7) We thank Dr. J. Walker (M.R.C., London) for an authentic specimen of *dl*-alaninol.

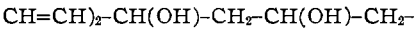
incorporation into the lactone, **3** may now be represented as the diacetate of **5**.



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



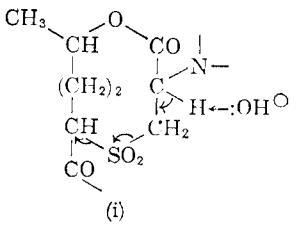
The precursor of alanine$_{(2)}$ in **5** has the following properties. It contains ethylenic unsaturation which gives rise to a band at 1625 cm.$^{-1}$ in the infrared. The terminal carbon atom bears a single hydrogen atom, and, since this is responsible for a singlet at τ 1.9, the carbon atom bearing 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.

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RECEIVED SEPTEMBER 4, 1962

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).