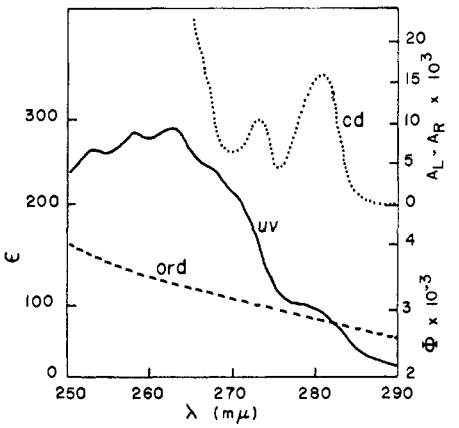

Figure 2. Ultraviolet, optical rotatory dispersion, and circular dichroism spectra of residual phenyl tetrahydrofurfuryl sulfite in acetonitrile. Optical path lengths: 1 cm. Concentration for CD spectrum: 4.25×10^{-3} *M*.

active.[10] Its infrared spectrum was identical with that of the original ester, which has no measurable optical rotation. The acid-catalyzed hydrolysis of the ester isolated after completion of the enzymic reaction can be followed spectropolarimetrically; the decrease in optical rotation follows first-order kinetics. The rotation was zero after 45 min at pH 0. These results provide convincing evidence that pepsin preferentially catalyzes the hydrolysis of one enantiomer of a sulfite ester.

The degree of stereospecificity exhibited in pepsin-catalyzed hydrolysis of alkyl phenyl sulfite esters is dependent on the structure of the alkyl group. The hydrolysis of methyl phenyl sulfite proceeds smoothly to 100% completion. The interpretation of this result is either that the small size of the methyl group may allow both enantiomers of this ester to form Michaelis complexes which undergo reaction at similar rates or that the less reactive enantiomer may racemize rapidly. The behavior of the phenyl cyclohexyl sulfite–pepsin system is intermediate, showing a distinctly biphasic reaction time course. Enzymic hydrolysis of this ester proceeds to 100% completion, one optical antipode reacting at least ten times faster than the other.

Figure 2 presents part of the circular dichroism spectrum of the unreacted phenyl tetrahydrofurfuryl sulfite ester isolated after enzymic hydrolysis.[11] The CD spectra from two independent experiments virtually superimpose. Interestingly the CD spectrum of recovered cyclohexyl ester is essentially identical; this suggests that the relative configuration of the more reactive enantiomer of each ester is probably the same. The structural requirements in the substrate which lead to stereospecific interactions with pepsin remain unanswered.

(10) For preparative runs conditions were the same as in Figure 1, curve 1. The reaction solution (250 ml) was extracted with 150 and 50 ml of dichloromethane after 45 min, corresponding to point X on curve 1. Combined dichloromethane extracts were washed with 5% aqueous sodium bicarbonate (three 30-ml portions), dried over magnesium sulfate–charcoal, and filtered. The solvent was removed under reduced pressure at 0°. The possibility that there was some contamination of the optically active sulfite ester by other optically active materials, *e.g.*, from degradation of the enzyme, is unlikely. First, control experiments showed that no extractable optically active material was formed in solutions containing enzyme only. Second, the acid-catalyzed hydrolysis of the extracted sulfite ester always resulted in complete loss of optical rotation.

(11) Circular dichroism studies were made on two Jasco-Durrum CD recording spectrophotometers made available to us through the kind cooperation of Dr. N. S. Simmons and Dr. W. F. H. Mommaerts. Both instruments show positive CD bands near 272 and 280 mμ, but the magnitude differs by 50% or less. The positive CD band(s) below 260 mμ are very large and have not been studied yet.

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Studies on Polypeptides. XXXVIII. Elimination of the Histidine Residue as an Essential Functional Unit for Biological Activity of β-Corticotropin[1-3]

Sir:

β-Corticotropin$_{1-20}$ amide (I),[4] a fragment of β-corticotropin[5] which in the rat possesses essentially the same *in vivo* adrenocorticotropic activity as the parent hormone,[6] was selected as a convenient molecule to investigate the effects of amino acid substitutions on biological activity.

In a previous study we synthesized 4-α-aminobutyric acid,5-glutamine-β-corticotropin$_{1-20}$ amide (II) and observed that this compound exhibited approximately 70% the biological activity of corticotropin A_1 on a weight basis.[7,8] From this result we concluded that the methionine sulfur is not essential for the biological function of ACTH. This conclusion was fully confirmed by Boissonnas, *et al.*,[9,10] who recorded the very high adrenocorticotropic potency of 625 IU/mg for 1-D-serine,4-norleucine,25-valine-β-corticotropin$_{1-25}$ amide.

We now wish to report the synthesis and some biological properties of 5-glutamine,6-β-(pyrazolyl-3)-alanine-β-corticotropin$_{1-20}$ amide (III).

The replacement of the histidine residue by β-(pyrazolyl-3)-alanine which results in a marked change in acid–base characteristics is not likely to alter significantly the conformation of peptide I since both imidazole and pyrazole are isosteric aromatic ring systems.[11]

In vivo corticotropic activity of homogeneous preparations of III was determined by three independent groups of investigators with essentially the same results.

The rat adrenal ascorbic acid depletion assay[12] showed compound III to possess approximately 50

(1) See F. M. Finn and K. Hofmann, *J. Am. Chem. Soc.*, **89**, 5298 (1967), for paper XXXVII in this series.

(2) The authors wish to express their appreciation to the U. S. Public Health Service for generous support of this investigation.

(3) Except for glycine the amino acid residues are of the L configuration. The following abbreviations are used: α-amino-*n*-butyric acid, Abut; β-(pyrazolyl-3)-alanine, Pyr(3)ala; DMF, dimethylformamide; DCC, dicyclohexylcarbodiimide; TFA, trifluoroacetic acid; AP-M, aminopeptidase M (G. Pfleiderer, P. G. Celliers, M. Stanulovic, E. D. Wachsmuth, H. Determann, and G. Braunitzer, *Biochem. Z.*, **340**, 552 (1964)). Thin layer chromatograms were performed in the systems 1-butanol–acetic acid–water 60:20:20 (R_f^{I}) and 1-butanol–pyridine–acetic acid–water 30:20:6:24 (R_f^{III}).

(4) K. Hofmann, H. Yajima, T.-Y. Liu, N. Yanaihara, C. Yanaihara, and J. L. Humes, *J. Am. Chem. Soc.*, **84**, 4481 (1962).

(5) R. G. Shepherd, K. S. Howard, P. H. Bell, A. R. Cacciola, R. G. Child, M. C. Davies, J. P. English, B. M. Finn, J. H. Meisenhelder, A. W. Moyer, and J. van der Scheer, *ibid.*, **78**, 5051 (1956).

(6) H. E. Lebovitz and F. L. Engel, *Endocrinology*, **75**, 831 (1964).

(7) K. Hofmann, J. Rosenthaler, R. D. Wells, and H. Yajima, *J. Am. Chem. Soc.*, **86**, 4991 (1964).

(8) H. B. F. Dixon and M. P. Stack-Dunne, *Biochem. J.*, **61**, 483 (1955).

(9) R. A. Boissonnas, St. Guttmann, and J. Pless, *Experientia*, **22**, 526 (1966).

(10) W. Doepfner, *ibid.*, **22**, 528 (1966).

(11) K. Hofmann and H. Bohn, *J. Am. Chem. Soc.*, **88**, 5914 (1966).

(12) Ascorbic depleting activity was determined in 24-hr hypophysectomized rats according to the method of "U. S. Pharmacopeia," Vol. XV, Mack Publishing Co., Easton, Pa., 1955, p 176, against the USP reference standard.

H-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-NH_2
I

H-Ser-Tyr-Ser-Abut-Gln-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-NH_2
II

H-Ser-Tyr-Ser-Met-Gln-Pyr(3)ala-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-NH_2
III

IU/mg. As concerns steroidogenic potency in the hypophysectomized or dexamethasone-blocked rat[13] peptide III exhibited approximately 60% the biological activity of corticotropin A_1 on a weight basis. Rat assays in which the steroidogenic potency of III was compared to the third International Standard gave a potency of more than 50 IU/mg. Accurate evaluation of potency was difficult because of nonparallelism in the log-dose response slopes of standard and peptide. A detailed account of the biological properties of the pyrazole analog will be published elsewhere.

In addition to its adrenocorticotropic activity peptide III exhibits *in vitro* melanocyte expanding activity (8.4×10^7 U/g).[14] This value is approximately 80% that recorded for peptide I, *i.e.*, 1.1×10^8 U/g.[4] On the basis of photolysis experiments, Dedman, *et al.*,[15] suggested that histidine may not be essential for function of ACTH. The present findings prove conclusively that the characteristic acid–base properties of the imidazole portion of histidine are not essential for both the adrenocorticotropic and melanocyte expanding properties of the β-corticotropin molecule.

For the synthesis of III, phenylalanylarginyltryptophylglycine[16] was acylated with *p*-nitrophenyl N^{α},N^{pyr}-dibenzyloxycarbonyl-β-(pyrazolyl-3)-alaninate [mp 156–157°; $[\alpha]^{24}D$ −17.0° (*c* 3.99, DMF). *Anal.* Found: C, 61.8; H, 4.4; N, 10.2] to give N^{α},N^{pyr}-dibenzyloxycarbonyl-β-(pyrazolyl-3)-alanylphenylalanylarginyltryptophylglycine which was converted by hydrogenolysis into β-(pyrazolyl-3)-alanylphenylalanylarginyltryptophylglycine [dihydrate $[\alpha]^{24}D$ −7.2° (*c* 1.90, 50% acetic acid); R_f^{I} 0.29; R_f^{III} 0.51; amino acid ratios in AP-M digest, $Pyr(3)ala_{0.91}Phe_{1.06}Arg_{0.99}Trp_{1.00}Gly_{1.05}$. *Anal.* Found: C, 55.0; H, 6.7; N, 20.6; O, 17.1].

The above pentapeptide was coupled with the azide of N-*t*-butoxycarbonylseryltyrosylserylmethionylglutamine [hydrazide hydrate mp 199–202°. *Anal.* Found: C, 48.5; H, 6.7; N, 15.1; O, 25.6] to give N-*t*-butoxycarbonylseryltyrosylserylmethionylglutaminyl-β-(pyrazolyl-3)-alanylphenylalanylarginyltryptophylglycine [monohydrate mp 200–203°; $[\alpha]^{27}D$ −15.4° (*c* 0.52, DMF); amino acid ratios in acid hydrolysate, $Ser_{1.88}Tyr_{0.92}Met_{0.95}Glu_{1.07}Pyr(3)ala_{1.02}Phe_{1.03}Arg_{1.16}Gly_{0.99}$; R_f^{I} 0.40; R_f^{III} 0.68, single Ehrlich- and chlorine-positive spot. *Anal.* Found: C, 54.6; H, 6.7; N, 17.1; O, 20.2].

The tosylate salt of this protected decapeptide was then coupled with the tosylate salt of N^{ϵ}-*t*-butoxycarbonyllysylprolylvalylglycyl-N^{ϵ}-*t*-butoxycarbonyllysyl-N^{ϵ}-*t*-butoxycarbonyllysylarginylarginylprolylvaline amide[7] using DCC as the condensing reagent. The ensuing protected eicosapeptide amide was isolated by chromatography on Sephadex G 25 [acetate, hydrate $[\alpha]^{27}D$ −50.4° (*c* 0.93, 10% acetic acid); R_f^{I} 0.46; R_f^{III} 0.65; amino acid ratios in acid hydrolysate, $Ser_{2.09}Tyr_{0.91}Met_{1.07}Glu_{1.00}Pyr(3)ala_{1.00}Phe_{1.01}Arg_{2.94}Gly_{2.02}Lys_{2.96}Pro_{2.00}Val_{2.05}$]. This material was deblocked by exposure to 90% TFA, trifluoroacetate ions were exchanged for acetate ions on Amberlite IRA-400, and the product was purified by chromatography on CMC.[17] Prior to assay the peptide was incubated with 2% aqueous thioglycolic acid[18] to reduce contaminating sulfoxide [acetate hydrate $[\alpha]^{27}D$ −62.1° (*c* 1.02, 10% acetic acid); R_f^{III} 0.52, single chlorine, ninhydrin; and Ehrlich-positive spot; single band on disc electrophoresis at pH 4.3; amino acid ratios in acid hydrolysate, $Ser_{2.16}Tyr_{0.91}Met_{0.98}Glu_{1.02}Pyr(3)ala_{0.92}Phe_{0.95}Arg_{2.95}Gly_{2.04}Lys_{3.00}Pro_{1.98}Val_{2.12}$; amino acid ratios in AP-M digest, $Ser_{1.99}Tyr_{0.92}Met_{1.03}Gln_{1.10}Pyr(3)ala_{0.82}Phe_{0.92}Arg_{2.87}Trp_{0.92}Gly_{2.17}Lys_{3.08}Pro_{2.14}Val_{2.16}$; peptide content 84% based on amino acid analysis].

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(17) E. A. Peterson and H. A. Sober, *J. Am. Chem. Soc.*, **78**, 751 (1956).
(18) K. Hofmann, F. M. Finn, M. Limetti, J. Montibeller, and G. Zanetti, *ibid.*, **88**, 3633 (1966).

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(13) R. Guillemin, G. W. Clayton, J. D. Smith, and H. S. Lipscomb, *Endocrinology*, **63**, 349 (1958).
(14) K. Shizume, A. B. Lerner, and T. B. Fitzpatrick, *ibid.*, **54**, 553 (1954).
(15) M. L. Dedman, T. H. Farmer, and C. J. O. R. Morris, *Biochem. J.*, **66**, 166 (1957).
(16) K. Hofmann and S. Lande, *J. Am. Chem. Soc.*, **83**, 2286 (1961).

Free-Radical Substitution on Adamantane

Sir:

Bridgehead radicals of less strained polycyclic compounds are said to be "normal"[1] for decarbonylation of RCO· (R is adamantyl-1 or bicyclo[2.2.2]oct-1-yl)[2] or photochlorination[3] and autoxidation[3] of adamantane. However, Stock concluded that the bridgehead (bicyclo-[2.2.2]-oct-1-yl) radical was unusually unstable from the observation that it readily abstracted chlorine from carbon tetrachloride or even from trichlorobromomethane in the presence of bromine.[4] Therefore, more work

(1) R. C. Fort, Jr., and P. von R. Schleyer, *Advan. Alicyclic Compounds*, **1**, 339 (1967).
(2) D. E. Applequist and L. Kaplan, *J. Am. Chem. Soc.*, **87**, 2194 (1965).
(3) G. W. Smith and H. D. Williams, *J. Org. Chem.*, **26**, 2207 (1961).
(4) F. W. Baker, H. D. Holtz, and L. M. Stock, *J. Org. Chem.*, **28**, 514 (1963).