spectral evidence supports structure VI rather than its metacyclophane valence tautomer, the delocalization energy of VI probably is in excess of that of two isolated benzene rings.

The synthesis of VI raises the possibility that a variety of such molecules can be prepared to test experimentally the exact nature of an aromatic π-electron cloud with regard to various physical and chemical properties such as steric hindrance, unusual bonding, and unusual interactions with ions or radicals generated within the π-electron cavity. Exploration of these general questions is being undertaken.[9]

(9) The analytical and spectral data of all of the compounds described are in accord with the assigned structures.

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STUDIES ON POLYPEPTIDES. XXVII. ELIMINATION OF THE METHIONINE RESIDUE AS AN ESSENTIAL FUNCTIONAL UNIT FOR *in vivo* ADRENOCORTICOTROPIC ACTIVITY[1-3]

Sir:

Exposure to hydrogen peroxide lowers markedly the adrenal-stimulating activity of pig corticotropin and incubation with thiols brings about essentially complete reactivation of oxidized material.[4-8] This phenomenon is also observed with the skin darkening hormones α- and β-MSH and with parathyroid peptides.[9-11] All these hormones contain methionine as the sole sulfur containing residue, and reversible oxidation of the thioether sulfur to the sulfoxide appears to provide the basis for the deactivation–reactivation behavior.[10,12]

The non-essential nature of the methionine residue as concerns melanocyte expanding activity follows from the observation that a family of peptides related to the N-terminus of the α-MSH sequence possesses the ability to darken frog skin although they do not contain methionine.[13]

In order to clarify the essential nature of the methionine residue for adrenocorticotropic activity, we synthesized the eicosapeptide amide seryltyrosylseryl-α-amino-*n*-butyrylglutamylhistidylphenylalanylarginyltryptophylglycyllysylprolylvalylglycyllysyllysylarginylarginylprolylvaline amide (I) and evaluated its *in vivo* adrenocorticotropic activity in the rat. Peptide I is the α-amino-*n*-butyric acid analog of the adrenocorticotropically fully active eicosapeptide amide which corresponds to the N-terminal half of the pig corticotropin molecule.[14] Evaluation of the adrenocorticotropic activity of three different batches of I by the rat adrenal ascorbic acid depletion method[15] gave values of 31.2 ± 4.2, 37.8 ± 6.6, 39.2 ± 7.2 and 48.4 ± 10.4 IU/mg. Of considerable practical importance is the finding that peptide I appears to exhibit 0.7 times the i.v. potency when administration is by the subcutaneous route; values of 32.4 ± 3.0, 29.4 ± 3.0, 24.3 ± 3 and 21.6 ± 3.3 were obtained in four assays. Preliminary studies have shown peptide (I) to exhibit 30–40% the *in vitro* and approximately 35% the *in vivo* steroidogenic activity of corticotropin A_1 on a weight basis.[16] The melanophoretic activity of I is 1.6×10^7 MSH units/g.[17]

These findings exclude the methionine residue as a "functionally active site"[18] for adrenocorticotropic activity and suggest that oxidation of the methionine sulfur lowers markedly the binding affinity of corticotropin and its biologically active fragments for the receptor with a corresponding decrease in biological activity. The fact that the sulfoxide or sulfone of a biologically active peptide containing methionine may possess a significantly lower physiological activity than the genuine material appears to provide little information regarding the "functional" importance of the methionine residue.

For the synthesis of I, N-*t*-butyloxycarbonylseryltyrosylseryl-α-amino-*n*-butyrylglutamine (II) (dihydrate; *Anal.* Found: C, 50.0; H, 6.9; N, 12.3; m.p. 146–148°; $[\alpha]^{27}D$ −28.6° in methanol; R_f^1 0.73[19]) was coupled with histidylphenylalanylarginyltryptophylglycine[20] *via* the azide to give N-*t*-butyloxycarbonylseryltyrosylseryl-α-amino-*n*-butyrylglutaminylhistidylphenylalanylarginyltryptophylglycine (III) (monoacetate trihydrate; *Anal.* Found: C, 53.4; H, 6.7; N, 15.9; $[\alpha]^{27}D$ −27.2° in 50% v./v. acetic acid; R_f^1 0.53; amino acid ratios in acid hydrolysate $ser_{2.09}tyr_{1.00}but_{1.09}glu_{0.94}his_{1.00}phe_{0.96}arg_{1.00}gly_{0.96}$). The acetate (III) was converted to the hydrochloride which was then treated with the hydrochloride of N^ϵ-*t*-butyloxycarbonyllysylprolylvalylglycyl-N^ϵ-*t*-butyloxycarbonyllysyl-N^ϵ-*t*-butyloxycarbonyllysylarginylarginylprolylvaline amide (triacetate hexahydrate; *Anal.* Found: C, 50.0; H, 8.2; N, 16.3; $[\alpha]^{27}D$ −71.8° in 10% v./v. acetic acid; R_f^1 0.68; amino acid ratios in acid hydrolysate $lys_{3.06}pro_{1.94}val_{2.02}gly_{1.00}arg_{1.98}$) using N,N′-carbonyldiimidazole[21] as the condensing reagent. The ensuing protected eicosapeptide amide *t*-butyloxycarbonylseryltyrosylseryl-α-amino-*n*-butyrylglutaminylhistidylphenylalanylarginyltryptophylglycyl-N^ϵ-*t*-butyloxycarbonyllysylprolylvalylglycyl-N^ϵ-*t*-butyloxycarbonyllysyl-N^ϵ-*t*-butyloxycarbonyllysylarginylarginylprolylvaline amide (IV) (triacetate hydrate; $[\alpha]^{26}D$ −54.7° in 10% v./v. acetic acid; single ninhydrin nega-

(1) The authors wish to express their appreciation to the U. S. Public Health Service and the National Science Foundation for generous support of this investigation.

(2) The peptides and peptide derivatives mentioned are of the L-configuration. In the interest of space conservation the customary L-designation for individual amino residues is omitted.

(3) See *J. Am. Chem. Soc.*, **85**, 833 (1963), for paper XXVI in this series.

(4) M. L. Dedman, T. H. Farmer and C. J. O. R. Morris, *Biochem. J.*, **59**, xii (1955).

(5) H. B. F. Dixon, *Biochim. Biophys. Acta*, **18**, 599 (1955).

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

(7) T. H. Farmer and C. J. O. R. Morris, *Nature*, **178**, 1465 (1956).

(8) M. L. Dedman, T. H. Farmer and C. J. O. R. Morris, *Biochem. J.*, **66**, 166 (1957).

(9) H. B. F. Dixon, *Biochim. Biophys. Acta*, **19**, 392 (1956).

(10) K. Hofmann and H. Yajima, in "Recent Progress in Hormone Research," G. Pincus, Ed., Vol. 18, Academic Press Inc., New York, N. Y., 1962, p. 41.

(11) H. Rasmussen and L. C. Craig, *ibid.*, p. 269.

(12) M. L. Dedman, T. H. Farmer and C. J. O. R. Morris, *Biochem. J.*, **78**, 348 (1961).

(13) See K. Hofmann, *Ann. Rev. Biochem.*, **31**, 213 (1962), for tabulation of a series of melanophoretically active peptides.

(14) (a) K. Hofmann, T. Liu, H. Yajima, N. Yanaihara, C. Yanaihara and J. L. Humes, *J. Am. Chem. Soc.*, **84**, 1054 (1962); (b) K. Hofmann, H. Yajima, T. Liu, N. Yanaihara, C. Yanaihara and J. L. Humes, *ibid.*, **84**, 4481 (1962)

(15) Ascorbic acid depleting activity was determined in 24-hr. hypophysectomized rats according to the method of "U. S. Pharmacopeia," Vol. XV, against the U.S.P. reference standard. We are indebted to Dr. Joseph D. Fisher of Armour Pharmaceutical Company, Kankakee, Illinois, for these biological determinations.

(16) We wish to express our gratitude to Drs. F. L. Engel and H. E. Lebovitz of Duke University Medical School for these assays.

(17) We wish to express our thanks to Dr. A. B. Lerner of Yale University School of Medicine, for the MSH assays which were performed according to the method of K. Shizume, A. B. Lerner and T. B. Fitzpatrick, *Endocrinol.*, **54**, 553 (1954).

(18) See ref. 13 for definition of this term.

(19) R_f^1 values refer to the Partridge system (S. M. Partridge, *Biochem. J.*, **42**, 238 (1948); R_f^2 values refer to the system 1-butanol, pyridine, acetic acid, water 30:20:6:24 (S. G. Waley and J. Watson, *ibid.*, **55**, 328 (1953)). With the latter system R_f values are expressed as multiples of the distance traveled by a histidine marker.

(20) (a) K. Hofmann, M. E. Woolner, G. Spühler and E. T. Schwartz, *J. Am. Chem. Soc.*, **80**, 1486 (1958); (b) K. Hofmann and S. Lande, *ibid.*, **83**, 2286 (1961).

(21) (a) H. A. Staab, *Ann. Chem.*, **609**, 75 (1957); (b) G. W. Anderson and R. Paul, *J. Am. Chem. Soc.*, **80**, 4423 (1958); (c) R. Paul and G. W. Anderson, *ibid.*, **82**, 4596 (1960).

tive Pauly, Sakaguchi and Ehrlich positive spot with R_f[1] 0.61; single component on paper electrophoresis at pH 3.5; amino acid ratios in acid hydrolysate $ser_{2.17}tyr_{0.96}but_{1.13}glu_{1.00}his_{1.04}phe_{1.09}arg_{3.04}gly_{1.91}lys_{2.80}pro_{2.60}val_{1.91}$) was exposed to the action of anhydrous trifluoroacetic acid for 20 min. at room temperature to give crude I which was purified by chromatography on carboxymethylcellulose[22]; ($[\alpha]^{28}D$ −61.7° in 10% v./v. acetic acid; single ninhydrin, Pauly, Sakaguchi and Ehrlich positive spot with R_f[2] 0.7 × his; single component on disk electrophoresis at pH 4.6; amino acid ratios in acid hydrolysate $ser_{1.97}tyr_{0.91}but_{0.94}glu_{1.02}his_{1.02}phe_{0.98}arg_{2.91}gly_{1.89}lys_{3.31}pro_{2.16}val_{1.89}$). The observation that leucine aminopeptidase digests of I contain glutamic acid and not glutamine[23] demonstrates that the glutamine amide group is susceptible to hydrolysis by trifluoroacetic acid. Schwyzer, *et al.*,[24] exposed N-*t*-butyloxycarbonylseryltyrosylserylmethionylglutaminylhistidylphenylalanylarginyltryptophylglycyl - N^{ϵ} - *t* - butyloxycarbonyllysylprolylvalylglycyl-N^{ϵ}-*t*-butyloxycarbonyllysyl-N^{ϵ}-*t*-butyloxycarbonyllysylarginylarginylproline-*t*-butyl ester to the action of trifluoroacetic acid and stated, without experimental support, that this treatment did not remove the amide group from the glutamine residue. Our own results indicate that the glutamine amide group in sequences related to the N-terminus of the corticotropins undergoes significant hydrolysis on exposure to trifluoroacetic acid.

Acknowledgment.—The skillful technical assistance of Mrs. Chizuko Yanaihara, Mrs. Maria Gunther, Miss Priscilla Holland and Mr. John Humes is gratefully acknowledged.

(22) E. A. Peterson and H. A. Sober, *J. Am. Chem. Soc.*, **78**, 751 (1956).

(23) The enzyme preparation used in these studies fails to show glutaminase activity.

(24) R. Schwyzer, W. Rittel, H. Kappeler and B. Iselin, *Angew. Chem.*, **72**, 915 (1960).

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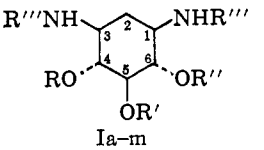
CHEMISTRY OF THE NEOMYCINS. XII[1] THE ABSOLUTE CONFIGURATION OF DEOXYSTREPTAMINE IN THE NEOMYCINS, PAROMOMYCINS AND KANAMYCINS

Sir:

The final uncertainty in the stereochemistry[1] of neomycin C, the absolute configuration of the unsymmetrically substituted deoxystreptamine portion of the antibiotic, now has been resolved.

N,N′-Diacetyl-6-O-methyldeoxystreptamine (Ib),[2-4] m.p. 280–284° dec., $[\alpha]^{23}D$ +5.0° (*c* 0.64, water), was prepared from poly-O-methyl-hexa-N-acetyl-neomycin B. This compound has been isolated previously from neomycin B (reported m.p. 282–284° dec., $[\alpha]^{27}D$ +12°),[2] from paromomycin (reported m.p. 280–282°, $[\alpha]^{27}D$ +15°),[3a] and from zygomycins A_1 and A_2 (reported m.p. 280–283°, $[\alpha]^{24}D$ +4°),[3b] which from their properties and degradation products are probably identical with paromomycin and paromomycin II,[2] respectively.



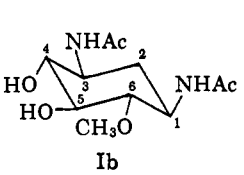
Ia–m

Ia (Deoxystreptamine): R = R′ = R″ = R‴ = H
Ib: R = R′ = H; R″ = CH_3; R‴ = $COCH_3$
Ic: R = CH_3; R′ = R″ = H; R‴ = $COCH_3$
Id: R = H; R′ = R″ = CH_3; R‴ = $COCH_3$

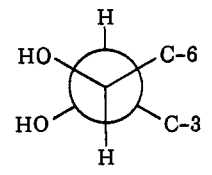
Ie (Neamine):	R = Neosamine C; R′ = R″ = R‴ = H
If (Neomycin C):	R = Neosamine C; R′ = Neobiosamine C; R″ = R‴ = H
Ig (Neomycin B):	R = Neosamine C; R′ = Neobiosamine B; R″ = R‴ = H
Ih (Paromomycin):	R = D-Glucosamine; R′ = Neobiosamine B; R″ = R‴ = H
Ii (Paromomycin II): (suggested)	R = D-Glucosamine; R′ = Neobiosamine C; R″ = R‴ = H
Ij (Paromamine):	R = D-Glucosamine; R = R″ = R‴ = H
Ik (Kanamycin C):	R = D-Glucosamine; R′ = R‴ = H; R″ = Kanosamine
Il (Kanamycin A):	R = 6-Aminoglucose; R′ = R‴ = H; R″ = Kanosamine
Im (Kanamycin B):	R = Diaminohexose; R′ = R‴ = H; R″ = Kanosamine

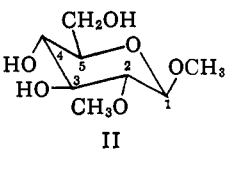
The work of Reeves[5] provides a tool for the determination of the stereochemical relationship between the remaining adjacent hydroxyl groups of Ib, by measuring the change in optical rotation, $\Delta[M]_{\text{Cupra B}}$, when cuprammonium hydroxide solution ("Cupra B") is substituted for water as the solvent.

The above compound (Ib) has $[\alpha]^{27}_{436}$ +9.8° (*c* 0.6, H_2O) and $[\alpha]^{27}_{436}$ +620° (*c* 0.47, Cupra B), giving $\Delta[M]_{\text{Cupra B}}$ = +1590. The high positive increment is similar to that observed with methyl 2-O-methyl-β-D-glucoside, II ($\Delta[M]_{\text{Cupra B}}$ = +2190) but of opposite sign to that of methyl 4-O-methyl-β-D-glucoside, III ($\Delta[M]_{\text{Cupra B}}$ = −2020).[6] Thus, the adjacent hydroxyl groups in 6-O-methyldeoxystreptamine are related as those at C-3 and C-4 of the glucopyranose ring,[7] *i.e.*, 6-O-methyldeoxystreptamine has structure Ib, rather than that of its mirror image Ic.

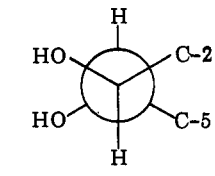


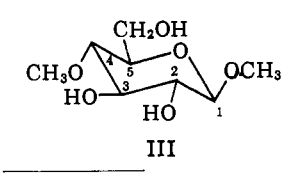
Ib



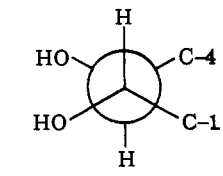


II





III



(1) Paper XI in this series: K. L. Rinehart, Jr., W. S. Chilton, M. Hichens and W. v. Phillipsborn, *J. Am. Chem. Soc.*, **84**, 3216 (1962).

(2) K. L. Rinehart, Jr., M. Hichens, A. D. Argoudelis, W. S. Chilton, H. E. Carter, M. Georgiadis, C. P. Schaffner and R. T. Schillings, *ibid.*, **84**, 3218 (1962).

(3) (a) T. H. Haskell, J. C. French and Q. R. Bartz, *ibid.*, **81**, 3483 (1959); (b) S. Horii, *J. Antibiotics* (Tokyo), *Ser. A*, **15**, 187 (1962).

(4) The numbering shown is that defined recently[2] for deoxystreptamine in neamine in order to place neosamine C on the lower numbered carbon. It is seen in the present report to have the additional advantage that it gives the lower number to the hydroxyl-bearing carbon (C-4) of absolute stereochemistry *R*.

(5) R. E. Reeves, *Advan. Carbohydrate Chem.*, **6**, 107 (1951).

(6) $\Delta[M]_{\text{Cupra B}} = ([\alpha]_{436}\ \text{Cupra B} - [\alpha]_{436}\ \text{water}) \times \frac{\text{mol. wt.}}{100}$.

(7) The possibility of unexpected interference by the acetamido groups in the cuprammonium complex is eliminated by the observation that crystalline N,N,-diacetyl-5,6-di-O-methyldeoxystreptamine (Id, sublimes above 260° without melting; *Anal.* Found: C, 52.70; H, 8.24; N, 10.68; OCH_3, 22.6), prepared from poly-O-methyl-tetra-N-acetylneamine, exhibited no enhanced rotation in Cupra B solution.