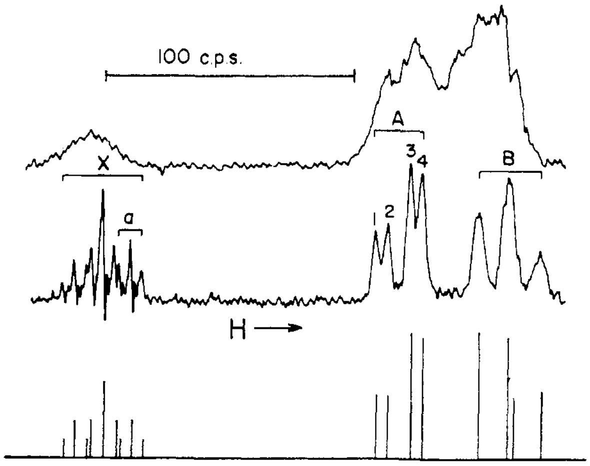

Fig. 1.—N.m.r. spectra (60 Mc./sec.) of cyclohexanol (top) and 3,3,4,4,5,5-hexadeuteriocyclohexanol (I) (middle) in D_2O. The OH band (actually of HDO) occurs at lower field and is not shown. The calculated spectrum (bottom) is that for an $(AB)_2X$ system with $\nu_{AB} = 38.0$, $J_{AB} = 12.2$, $J_{AX} = 4.09$ and $J_{BX} = 10.17$ cps. The calculated intensities of the X lines are not on the same scale as the AB lines.

In order to obtain spectra in which the bands of H1 consist of sharp lines, we have prepared[6] 3,3,4,4,5,5-hexadeuteriocyclohexanol (I), its acetate (II), and *cis*- and *trans*-3,3,4,5,5-pentadeuterio-4-*t*-butylcyclohexanol (III and IV, respectively).

In each of the above compounds (I-IV) the band of H1 in the n.m.r. spectrum consisted of a number of sharp well-resolved lines (*e.g.*, Fig. 1). The methylene protons, although somewhat broadened by coupling with deuterium atoms, were readily recognizable as the AB part of an ABX system. The band of H1 is the X part of two ABX systems. Analysis of the spectra gave the data in Table I.

TABLE I

VICINAL COUPLING CONSTANTS[a] OF CYCLOHEXANE DERIVATIVES

Compound[b]	State	$J_{a,a}$	$J_{a,e}$	$J_{e,e}$
IIA	25% in CS_2	11.43	4.24	
IIB	at −110°		(2.71)[c]	(2.71)[c]
III	8% in CCl_4		3.00	2.72
IV	10% in CC14	11.07	4.31	
		J_{large}	J_{small}	K[d]
I	5% in D_2O	10.17	4.09	8.09[e]
I	25% in CCl_4	9.77	3.93	5.25[e]
II	25% in CS_2	9.26	3.89	3.00[f]

[a] The coupling constants are considered to be accurate to better than 0.1 cps. [b] IIA and IIB are the equatorial-acetoxy and axial-acetoxy conformations of II; the ratio of IIA to IIB is about 10 to 1 at −110°. [c] Average of $J_{a,e}$ and $J_{e,e}$. [d] K = [equatorial OH conformer]/[axial OH conformer] and is calculated on the basis of K_{large}. [e] Calculated using the coupling constants found for III and IV above. [f] Calculated using the coupling constants found for IIA and IIB above.

It is surprising that the *gauche* coupling constants in IIA and IV are much larger than in IIB and III. It does not seem likely that changes in dihedral angles could be entirely responsible for this. On the whole, however, the values of the coupling constants are very much as expected.[1,2,5,7]

The relative signs of the coupling constants were determined for I in D_2O. From the relative intensities (2 > 1, 3 > 4) of the lines of the A spectrum (Fig. 1), it follows that J_{AX} and J_{BX} have the same sign. As anticipated[8,9] J_{AB} is of opposite sign to J_{BX} because the *high-field* triplet (a) of X collapsed to a single line when A was simultaneously irradiated[10] with a magnetic field of 1.5 milligauss at a frequency corresponding to the center of the *low-field* doublet of A.

It is possible, once accurate coupling constants are available for III and IV, to deduce conformational equilibria for I under various conditions. From the data in Table I the *A* value[11] of I is calculated to be 1.25 kcal./mole in D_2O and 1.0 kcal./mole in CCl_4 at 28°. By using the coupling constants found at low temperatures the *A* value of II in CS_2 at 28° is found to be 0.66 kcal./mole. The present *A* values are thought to be reliable, although they are somewhat higher than some of those previously reported.[11,12]

Measurements are being undertaken of the coupling constants of I, III, IV and their derivatives and of related compounds at different temperatures and in various solvents.

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(6) The reasons for using the particular deuterated derivatives and the method used for their syntheses will be discussed in a full paper. See also F. A. L. Anet, *Can. J. Chem.*, **39**, 2262 (1961).

(7) J. I. Musher, *J. Chem. Phys.*, **34**, 595 (1961).

(8) R. R. Fraser, R. U. Lemieux and J. D. Stevens, *J. Am. Chem. Soc.*, **83**, 3901 (1961).

(9) F. Kaplan and J. D. Roberts, *ibid.*, **83**, 4666 (1961).

(10) The method is that of D. F. Evans and J. P. Maher, *Proc. Chem. Soc.*, 208 (1961), and R. Freeman and D. H. Whiffen, *Mol. Phys.*, **4**, 321 (1961). The spin-decoupling technique of R. Kaiser, *J. Sci. Inst.*, **31**, 963 (1960), was used and the side band frequency required for the experiment described was 90 cps. The effect of other frequencies also was found to be consistent only with J_{AB} of opposite sign to J_{BX}.

(11) S. Winstein and J. N. Holness, *J. Am. Chem. Soc.*, **77**, 5562 (1955).

(12) E. L. Eliel, *J. Chem. Educ.*, **37**, 126 (1960), D. S. Noyce and L. J. Dolby, *J. Org. Chem.*, **26**, 3619 (1961), G. Chiurdoglu and W. Masschelein, *Bull. Soc. Chim. Belg.*, **69**, 154 (1960).

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STUDIES ON POLYPEPTIDES. XXII. HIGH ADRENOCORTICOTROPIC ACTIVITY IN THE RAT AND IN MAN OF A SYNTHETIC EICOSAPEPTIDE AMIDE[1-3]

Sir:

In 1956 Boissonnas, *et al.*,[4] announced a synthesis of the eicosapeptide (I) and reported that their preparation possessed low *in vitro* adrenocorticotropic activity (2 to 3 U./mg.). Recently, Li, *et al.*,[5] described a preparation of the nonadecapeptide (II) which exhibited *in vivo* adrenocorticotropic

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(1) Supported by grants from the U. S. Public Health Service, the National Science Foundation and the American Cancer Society.

(2) Peptides and peptide derivatives mentioned in this communication are of the L-variety.

(3) See *J. Am. Chem. Soc.*, **83**, 2294 (1961), for paper XXI in this series.

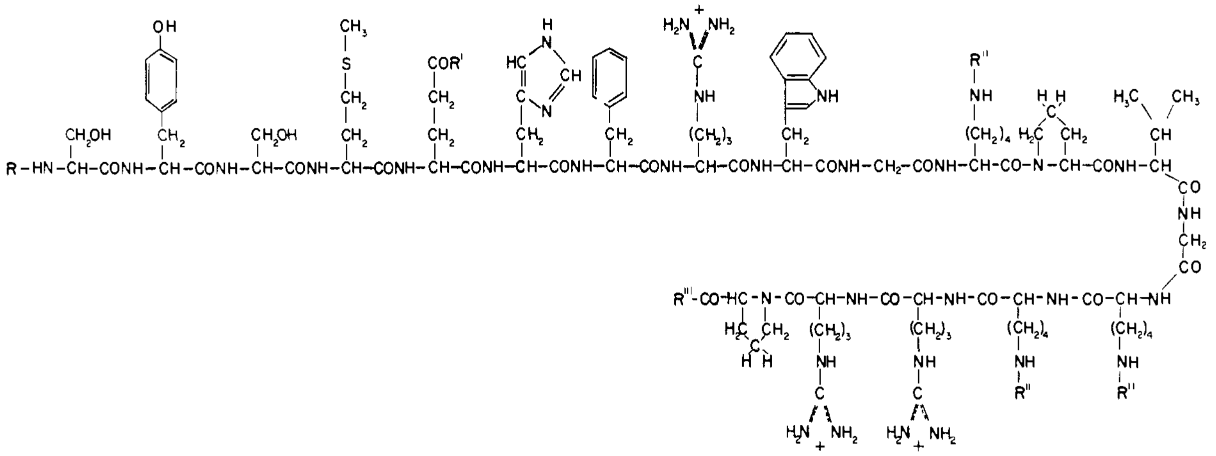
(4) R. A. Boissonnas, St. Guttmann, J.-P. Waller and P.-A. Jaquenoud, *Experientia*, **12**, 446 (1956).

(5) C. H. Li, J. Meienhofer, E. Schnabel, D. Chung, T. Lo and J. Ramachandran, *J. Am. Chem. Soc.*, **83**, 4449 (1961).

activity at the level of 35 U./mg. and its glutamine analog (III), according to Schwyzer, *et al.*,[6] possesses essentially the same potency. Since it appeared rather unlikely that these shorter chain peptides should possess a higher level of biological activity than (I), we prepared the eicosapeptide amide (IV) and evaluated its biological potency both in the rat and in man. The most highly purified samples of our preparation possessed essentially the same biological activity as natural ACTH. This communication summarizes our pertinent findings.

FORMULA

	R	R′	R″	R‴
(I)	H	OH	H	val
(II)	H	OH	H	OH
(III)	H	NH_2	H	OH
(IV)	H	H	H	val amide
(V)	Acetyl	NH_2	Formyl	val amide



Arginylarginylprolylvaline amide (triacetate tetrahydrate; *anal.* found: C, 43.7; H, 8.2; N, 19.8; $[\alpha]^{31}_{\mathrm{D}}$ −40.2° in 10% acetic acid; R_f,[7] 0.39; ninhydrin, and Sakaguchi positive spot; amino acid ratios in acid hydrolysate $\mathrm{arg}_{2.00}\mathrm{pro}_{0.99}\mathrm{val}_{1.01}$; amino acid ratios in leucine aminopeptidase (LAP) digest[8] $\mathrm{arg}_{2.01}\mathrm{pro}_{0.96}\mathrm{val}_{1.00}$) was synthesized from prolylvaline amide[9] by the stepwise nitroarginine procedure.[10] This tetrapeptide amide was coupled with N^{α}-carbobenzoxy-N^{ϵ}-formyllysylprolylvalylglycyl-N^{ϵ}-formyllysyl-N^{ϵ}-formyllysine azide[10] to give a protected decapeptide amide which was decarbobenzoxylated by hydrogenolysis to N^{ϵ}-formyllysylprolylvalylglycyl-N^{ϵ}-formyllysyl-N^{ϵ}-formyllysylarginylarginylprolylvaline amide (triacetate octahydrate; *anal.* found: C, 46.5; H, 8.1; N, 18.2; $[\alpha]^{30}_{\mathrm{D}}$ −73.5° in 10% acetic acid; R_f[7] 0.60, ninhydrin and Sakaguch, positive spot; amino acid ratios in acid hydrolysatei $\mathrm{lys}_{3.02}\mathrm{pro}_{2.02}\mathrm{val}_{2.04}\mathrm{gly}_{1.07}\mathrm{arg}_{1.80}$; amino acid ratios in LAP digest,[8] $\mathrm{lys}_{3.03}\mathrm{pro}_{2.07}\mathrm{val}_{2.03}\mathrm{gly}_{0.97}\mathrm{arg}_{1.93}$). The protected eicosapeptide amide (V) (triacetate tetradecahydrate; *anal.* found: C, 50.1; H, 7.0; N, 17.1; $[\alpha]^{28}_{\mathrm{D}}$ −56.0° in 10% acetic acid; R_f[11] 0.34, ninhydrin negative, Pauly, methionine, Ehrlich and Sakaguchi positive spot; amino acid ratios in acid hydrolysate $\mathrm{ser}_{2.08}\mathrm{tyr}_{0.97}\mathrm{met}_{0.93}\mathrm{glu}_{1.04}\mathrm{his}_{1.00}\mathrm{phe}_{1.00}\mathrm{arg}_{2.85}\mathrm{gly}_{1.96}\mathrm{lys}_{2.92}\mathrm{pro}_{2.04}\mathrm{val}_{1.96}$) was prepared by treating N-acetylseryltyrosylserylmethionylglutaminylhistidylphenylalanylarginyltryptophylglycine[12] and the trihydrochloride of the above-described partially protected decapeptide amide with N,N′-dicyclohexylcarbodiimide[13] serving as the condensing reagent. The protected eicosapeptide amide (V) exhibited melanophoretic activity of 4.2 × 10^8 MSH U./g.[14]

Exposure of (V) for 60 to 80 minutes to 0.5 *N* hydrochloric acid at the temperature of a boiling water-bath gave a mixture of compounds which possessed adrenal ascorbic acid depleting activity (30–35 U./mg.).[15] Highly purified samples of the eicosapeptide amide (IV) ($[\alpha]^{26}_{\mathrm{D}}$ −56.8° in 10% acetic acid; R_f[7] 1.35 × his; ninhydrin, Pauly, methionine, Ehrlich and Sakaguchi positive spot; homogeneous on paper electrophoresis at pH 3.8, 5.1 and 6.9 and also on starch-gel electrophoresis at pH 8.5; amino acid ratios in acid hydrolysate $\mathrm{ser}_{1.58}\mathrm{tyr}_{0.75}\mathrm{met}_{0.96}\mathrm{glu}_{0.96}\mathrm{his}_{1.00}\mathrm{phe}_{1.04}\mathrm{arg}_{3.04}\mathrm{gly}_{2.13}\mathrm{lys}_{3.25}\mathrm{pro}_{2.13}\mathrm{val}_{2.13}$; amino acid ratios in LAP digest $\mathrm{ser}_{1.50}\mathrm{tyr}_{0.87}\mathrm{met}_{1.04}\mathrm{glu}_{1.10}\mathrm{his}_{1.04}\mathrm{phe}_{0.93}\mathrm{arg}_{1.45}\mathrm{try}_{0.99}\mathrm{gly}_{1.45}\mathrm{lys}_{3.43}\mathrm{pro}_{1.45}\mathrm{val}_{1.40}$[16]; C-terminal valine not released by

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

(7) Solvent system 1-butanol, pyridine, water, acetic acid, 30:20:24:6, S. G. Waley and J. Watson, *Biochem. J.*, **55**, 328 (1953).

(8) Peptides containing arginylproline and lysylproline bonds are completely digestible by our crude LAP preparation but proline was not liberated by a commercial preparation (Worthington) of the enzyme.

(9) K. Hofmann, T. A. Thompson, M. E. Woolner, G. Spühler, H. Yajima, J. D. Cipera and E. T. Schwartz, *J. Am. Chem. Soc.*, **82**, 3721 (1960).

(10) K. Hofmann, T. Liu, H. Yajima, N. Yanaihara and S. Lande, *ibid.*, **83**, 2294 (1961).

(11) S. M. Partridge, *Biochem. J.*, **42**, 238 (1948).

(12) K. Hofmann and H. Yajima, *J. Am. Chem. Soc.*, **83**, 2289 (1961).

(13) J. C. Sheehan and G. P. Hess, *ibid.*, **77**, 1067 (1955).

(14) We wish to express our thanks to Drs. A. B. Lerner and J. S. McGuire of the Department of Medicine, Yale University School of Medicine, for the MSH assays, which were carried out according to the procedure of Shizume, Lerner and Fitzpatrick, *Endocrinol.*, **54**, 553 (1954).

(15) Ascorbic acid depleting activity was determined in 24-hour hypophysectomized rats according to the method of U. S. Pharmacopoeia XV against the USP reference standard. We are much indebted to Dr. Joseph D. Fisher of Armour Pharmaceutical Company, Kankakee, Illinois, for these assays.

(16) The low recovery of arginine and high lysine value are the result of ornithine formation; see ref. 10.

carboxypeptidase; MSH activity 1.1 × 10^8 U./g.[15]) were isolated from the partial hydrolysate by chromatography on carboxymethylcellulose,[17] cellulose-block electrophoresis at pH 7.0 and then c.m.c. chromatography.

Repeated assays of various batches of our preparation of the eicosapeptide amide by the rat adrenal ascorbic acid depletion method gave an average value of 111.0 ± 18.0 U./mg. For determination of the ability of the material to bring about steroidogenesis in man the preparation was infused over a 15 minute period and plasma 17-hydroxysteroid levels were determined at 0, 15, 30 and 60 minutes. The material was administered to 22 patients, and at all levels above 5 units there was a significant rise in plasma 17-hydroxysteroids which occurred maximally at 30 minutes but persisted into the 60 minute period.[18]

In conjunction with previous observations these findings indicate that the structural elements which are essential for full adrenocorticotropic activity reside in that portion of the ACTH molecule which corresponds to the first twenty residues from the amino end. It is of interest that the addition of the valine amide moiety to the C-terminus of the nonadecapeptide (II) brings about such a remarkable increase in biological potency. We have no explanation for the discrepancy between our results and those of Boissonnas, *et al.*[4]

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

[18] We wish to express our sincere thanks to Dr. H. S. Lipscomb, Department of Physiology, Baylor University College of Medicine for the results with human patients which will be presented in a separate publication.

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DERIVATIVE CHEMISTRY OF $B_{10}H_{10}^{=}$ AND $B_{12}H_{12}^{=}$

Sir:

We wish to report a derivative chemistry of such scope and diversity as to presage a new major area of inorganic chemistry. This chemistry is based on reactions of the polyhedral borane anions $B_{10}H_{10}^{=}$ and $B_{12}H_{12}^{=}$.[1,2] The stability of these anions to strong base, strong acids and oxidizing agents is unique for boron hydride structures. For example, they are inert to hot sodium hydroxide or sodium methoxide solutions and can be converted to the stable acids $(H_3O)_2B_{10}H_{10}\cdot xH_2O$ and $(H_3O)_2B_{12}H_{12}\cdot xH_2O$. These acids are slightly stronger than sulfuric acid.[3] The anions form insoluble, apparently covalent silver salts with no reduction to silver metal. However, the anions react smoothly with certain reagents, particularly electrophilic species, to give stable derivatives in which hydrogen atoms are replaced by the attacking group.

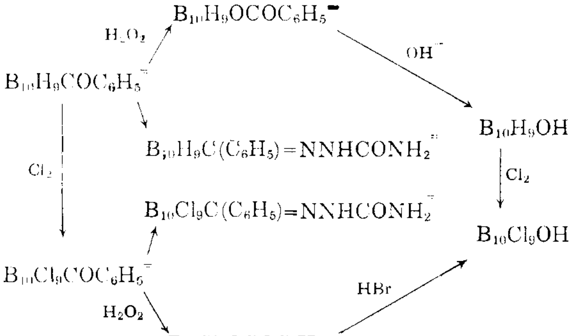
All of the halogens react smoothly with aqueous or alcoholic solutions of $B_{10}H_{10}^{=}$ and $B_{12}H_{12}^{=}$ to give a series of haloborates. Examples of some of the anions we have isolated in the form of salts and crystalline hydrated acids[4] are $B_{10}Cl_{10}^{=}$, $B_{12}H_3Br_6Cl_3^{=}$, $B_{12}F_{12}^{=}$, $B_{12}I_{12}^{=}$, $B_{10}H_3Br_7^{=}$, $B_{10}H_6I_4^{=}$, $B_{12}H_{11}I^{=}$, $B_{12}Cl_{10}H_2^{=}$, $B_{12}Br_{12}^{=}$ and $B_{10}I_{10}^{=}$. The halogenated salts have excellent chemical and thermal stabilities. For example, differential thermal analysis studies have shown that $Cs_2B_{10}Cl_{10}$ is stable to at least 400° in the presence of air. The halogen–boron bonds are inert to nucleophiles such as hydroxide or methoxide in refluxing water or methanol.

Anal. Calcd. for $[(CH_3)_4N]_2B_{12}Br_{12}$: C, 7.8; H, 2.0; B, 10.5; Br, 77.6. Found: C, 8.3; H, 2.3; B, 10.6; Br, 77.7. Calcd. for $(H_3O)_2B_{10}I_{10}\cdot 6H_2O$: B, 7.1; I, 83.5; neut. equiv., 760. Found: B, 7.0; I, 83.5; neut. equiv., 754.

$B_{12}H_{12}^{=}$ has been nitrated with nitric acid under carefully controlled conditions to give $B_{12}H_{11}NO_2^{=}$.

Anal. Calcd. for $Cs_2B_{12}H_{11}NO_2$: B, 28.7; H, 3.4; N, 3.1. Found: B, 28.3; H, 3.1; N, 3.4. The presence of the nitro group is confirmed by infrared absorption bands at 6.35 μ and 7.7 μ.

Benzoyl chloride reacts with $(H_3O)_2B_{10}H_{10}$ to give $B_{10}H_9COC_6H_5^{=}$ which can undergo further reactions at either the B–H or the carbonyl functionalities.



Anal. Calcd. for $[(CH_3)_3S]_2B_{10}Cl_9C(C_6H_5){=}NNHCONH_2$: C, 22.5; H, 3.5; B, 14.5; N, 5.6; Cl, 43.0. Found: C, 21.7; H, 3.4; B, 15.0; N, 4.9; Cl, 44.2. Calcd. for $[(CH_3)_4N]_2B_{10}Cl_9OH$: C, 16.2; H, 4.2; B, 18.3; Cl, 53.9. Found: C, 16.8; H, 4.7; B, 18.3; Cl, 53.2.

The carbonyl absorption in the infrared spectra of $B_{10}H_9COC_6H_5^{=}$ and $B_{10}Cl_9COC_6H_5^{=}$ is shifted to about 6.4 μ and its intensity decreased so that it is difficult to distinguish from the phenyl bands. The preparation of semicarbazones demonstrates that carbonyl reactivity persists, however. Oxidation with hydrogen peroxide to $B_{10}H_9OCOC_6H_5^{=}$ and $B_{10}Cl_9OCOC_6H_5^{=}$ restores normal carbonyl intensity and the position of the carbonyl band drops back to 6.0 μ.

Amides also react with concentrated aqueous solutions of $B_{10}H_{10}^{=}$ under acid conditions. The

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[1] These anions may be called decahydrodecaborate(-2) and dodecahydrododecaborate(-2) following proposals made by the Committee on Nomenclature of the American Chemical Society, Division of Inorganic Chemistry; *cf.* paper by K. L. Loening to the Division of Chemical Literature, 134th American Chemical Society Meeting, Chicago, Illinois, September, 1958, abstracts, p. 1-G.

[2] M. F. Hawthorne and A. R. Pitochelli, *J. Am. Chem. Soc.*, **81**, 5519 (1959); M. F. Hawthorne and A. R. Pitochelli, *ibid.*, **82**, 3228 (1960).

[3] Y. T. Chia, to be published.

[4] These are strong acids; in general comparable to sulfuric acid.